PB87-112322

Problems of Aquatic Toxicology
Biotesting and Water Quality Management
Proceedings of USA-USSR Symposium
Borok, Jaroslavl Oblast, July 30-August 1, 1984

Environmental Research Lab., Athens, GA

Aug 86

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Athens GA 30613

EPA/600/9-86/024
August 1986

Research and Development

PB87-112322

EPA

Problems of Aquatic Toxicology, Biotesting and Water Quality Management:

Proceedings of USA-USSR Symposium, Borok, Jaroslavl Oblast, July 30-August 1, 1984

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/9-86/024	2.	3. RECIPIENT'S ACCESSION NO. PB87 112322/AS
4. TITLE AND SUBTITLE PROBLEMS OF AQUATIC TOXICOLOGY, BIOTESTING, AND WATER QUALITY MANAGEMENT: proceedings of USA-USSR Symposium, Borok, Jaroslavl Oblast, July 31-August 1, 1984		5. REPORT DATE August 1986
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory U.S. Environmental Protection Agency Athens, GA 30613		10. PROGRAM ELEMENT NO. ANWB1E
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Athens, GA Office of Research and Development U.S. Environmental Protection Agency Athens, GA 30613		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/600/01

15. SUPPLEMENTARY NOTES

Edited by Robert C. Ryans

16. ABSTRACT

Sixteen papers delivered by USA and USSR scientists at a symposium entitled "Problems of Aquatic Toxicology, Biotesting, and Water Quality Management" are presented. The effect of low pH on aquatic invertebrates and the use of fish behavior in comparing toxic effects of chemicals are examined. The production and excretion of ammonia by fish, the relationship between carbon dioxide excretion and ammonia toxicity, and the acute toxicity of iron cyanides and thiocyanate to trout are discussed. Mechanisms of organophosphorus pesticide resistance are analyzed. Biological monitoring and testing of surface waters and effluent is discussed and a complex effluents toxicity information system is described. Processes in the formation of water quality and trends in the development of water' quality in the two countries are outlined. Pollution problems from toxic organic contaminants from point and nonpoint sources in the North American Great Lakes are examined. Agricultural water quality management, including the use of simulation models, is discussed.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 238
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/9-86/024

PROBLEMS OF AQUATIC TOXICOLOGY, BIOTESTING AND WATER QUALITY MANAGEMENT:
Proceedings of USA-USSR Symposium, Borok, Jaroslavl Oblast, July 30-August 1, 1984

Edited by
Robert C. Ryans

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30613

DISCLAIMER

The information in this document has been funded in part by the United States Environmental Protection Agency. Papers describing EPA-sponsored research have been subject to the Agency's peer and administrative review, and the proceedings have been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

FOREWORD

For almost a decade and a half, cooperation and exchange of scientific information under the US-USSR Agreement on Cooperation in the Field of Environmental Protection has helped both countries in their efforts to control environmental pollution. These efforts are pursued in recognition of the international nature of the problem: pollution knows no boundaries.

Three projects are under the joint US-USSR Agreement's Working Group on Cooperation in the Area of Water Pollution Prevention. These are: Project 02.02-11 "Planning and Management of Water Quality in River Basins," Project 02.02-12 "Protection and Management of Water Quality in Lakes and Estuaries," and Project 02.02-13 "Effect of Pollutants on Aquatic Organisms and Ecosystems; Development of Water Quality Criteria."

Over the years, scientific delegations and individual scientists have traveled to each other's countries to visit scientific institutions, perform joint research, and exchange technical information. This Proceedings presents the papers that were delivered at the most recent formal symposium, which was held in Borok, Jaroslavl Oblast in 1984.

Rosemarie C. Russo
Director
Environmental Research Laboratory
Athens, Georgia
USA

PREFACE

The problem of water pollution and the control of water quality has assumed international importance due to its urgency. The pooling of efforts by scientists from different countries has helped to achieve successful solutions. Within the context of the Soviet-American agreement in the area of environmental conservation, provision has been made both for the carrying out of joint research and the holding of symposia at which specialists would be able to discuss results and outline prospects for further work.

A regular Soviet-American symposium on the questions of water toxicology was held in the USSR at the Institute for the Biology of Inland Waters Under the USSR Academy of Sciences in Borok from 30 July through 1 August 1984. At the symposium, eight Soviet and eight American papers were presented and the materials of these are being published in the current collection. These papers reflect the accomplishments of aquatic toxicology at the present stage of its development and examines the following: water quality in natural waters, question of utilizing indicators for the functional state of biota in surface waters, approaches to biotesting of industrial effluents, and principles of optimizing development programs for water conservation in the system of water quality management considering point and non-point pollution sources.

Of substantial interest are the results of studying the effect of complex-composition effluents on hydrobionts, the resistance of aquatic animals to organophosophorus pesticides and the possibilities for further transfer of toxic organic substances. The spread of pollutants through the atmosphere in a number of instances has substantial negative ecological consequences and the study of this process is of important significance. In light of this problem, within the context of Soviet-American collaboration, particular attention is being paid to studying ammonia toxicity for fish as well as to the effect of low pH values on aquatic animals.

The symposium, which was held in a spirit of mutual understanding, showed that the mutual interest of the United States and the USSR in studying the problem of protecting bodies of water against pollution has successfully contributed in this area to the solving of problems confronting scientists from both countries.

N. V. Butorin, Director
Institute for Biology of Inland Waters
Borok, Jaroslavl Oblast
USSR

ABSTRACT

Sixteen papers delivered by US and USSR scientists at a symposium entitled "Problems of Aquatic Toxicology, Biotesting, and Water Quality Management" are presented. The effect of low pH on aquatic invertebrates and the use of fish behavior in comparing toxic effects of chemicals are examined. The production and excretion of ammonia by fish, the relationship between carbon dioxide excretion and ammonia toxicity, and the acute toxicity of iron cyanides and thiocyanate to trout are discussed. Mechanisms of organophosphorus pesticide resistance are analyzed. Biological monitoring and testing of surface waters and effluent is discussed and a complex effluents toxicity information system is described. Processes in the formation of water quality and trends in the development of water quality in the two countries are outlined. Pollution problems from toxic organic contaminants from point and non-point sources in the North American Great Lakes are examined. Agricultural water quality management, including the use of simulation models, is discussed.

CONTENTS

FOREWORD.. iii

PREFACE... iv

ABSTRACT.. v

FUNCTIONAL BASES FOR THE EFFECT OF LOW pH ON FISH AND INVERTEBRATES.. 1
G. A. Vinogradov

AMMONIA PRODUCTION AND EXCRETION BY FISH.................... 19
D. J. Randall and P. A. Wright

THE USE OF FISH BEHAVIOR IN COMPARING TOXIC EFFECTS OF THREE CHEMICALS.. 31
M. G. Henry

RESISTANCE OF AQUATIC ANIMALS TO ORGANOPHOSPHORUS PESTICIDES AND ITS MECHANISMS.. 37
V. I. Kozlovskaya, G. M. Chuyko, L. N. Lapkina, and V. A. Nepomnyashchikh

ACUTE TOXICITY OF IRON CYANIDES AND THIOCYANATE TO TROUT..... 55
R. V. Thurston and T. A. Heming

PROCESS OF FORMATION OF NATURAL WATER QUALITY............... 72
M. I. Kuz'menko and A. I. Merezhko

CO_2 EXCRETION AND AMMONIA TOXICITY IN FISHES: IS THERE A RELATIONSHIP?.. 83
T. A. Heming

USE OF INDICATORS OF FUNCTIONAL STATE OF BIOTA IN BIOLOGICAL MONITORING OF SURFACE WATERS............................... 95
V. A. Bryzgalo, L. S. Federova, T. A. Khoruzhaya, L. S. Kosmenko, and L. P. Sokolova

LONG RANGE TRANSPORT OF TOXIC ORGANIC CONTAMINANTS TO THE NORTH AMERICAN GREAT LAKES................................. 107
W. R. Swain, M. D. Mullin, and J. C. Filkins

BIOLOGICAL TESTING OF INDUSTRIAL EFFLUENT................... 122
A. M. Beym

COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM.......................... 136
R. C. Russo, A. Pilli, and J. Crane

OPTIMIZING A PROGRAM FOR THE DEVELOPMENT OF WATER CONSERVATION IN A SYSTEM OF WATER QUALITY MANAGEMENT CONSIDERING POINT AND NON-POINT POLLUTION SOURCES.................................. 150
G. A. Sukhorukov

GREAT LAKES AGRICULTURAL POLLUTION CONTROL IN PERSPECTIVE.. 168
V. J. Saulys

MODELING THE FORMATION OF WATER QUALITY IN WATER CHANNELS RECEIVING SURFACE RUNOFF FROM FARM LAND..................... 181
Ye. V. Yeremenko, V. Z. Kolpak and N. I. Selyu

CONTROL OF URBAN NONPOINT SOURCES IN GREAT LAKES BASIN.................. 201
D. Athayde, P. Bubar, and J. Meek

TRENDS IN THE DEVELOPMENT OF WATER QUALITY IN THE USSR AND THE US.. 219
L. A. Lesnikov

ACKNOWLEDGMENT

Preparation of a Proceedings is frequently a complex task, particularly when the authors are from two countries with different languages. Of great assistance in the preparation of this Proceedings was the contribution of Dr. Richard A. Schoettger of the Fish and Wildlife Service's Columbia National Fisheries Research Laboratory who arranged for the translation of the Soviet manuscripts. Also essential to its preparation was the dedicated work of Martha M. Brady of Computer Sciences Corporation who typed the final document. Their contributions are gratefully acknowledged.

FUNCTIONAL BASES FOR THE EFFECT OF LOW pH ON FISH AND INVERTEBRATES

by

G.A. Vinogradov[1]

ABSTRACT

Low, acutely toxic pH levels increase the ion permeability of gill epithelium in fish and invertebrates. As a result of the increased permeability, the rate of salt diffusion from the organism increases by several fold. Electrolyte concentration in the blood drops rapidly, there is an intense intake of hydrogen ions according to the concentration gradient into the inner medium, and acidosis develops in parallel with salt elimination. In fish with low pH values, there are destructive changes in the gill epithelium and these develop most intensely in a medium containing less than 20 mg/ℓ Ca^{++}. The pH values that cause substantial changes in gill epithelium permeability for Na^+ correlate with low limits of pH values that are encountered by adult specimens of various fish and invertebrate species under natural conditions. Two adaptive processes occur simultaneously in fish in acclimation to low pH values. On the one hand, as a consequence of the reduced gill permeability, the elimination of Na^+ and Cl^- from the organism is reduced; on the other, after a significant suppression of the absorption of Na^+ and Cl^-, a partial recovery of this process is observed. As a result of acclimation, after 24-48 h, the losses and absorption of Na^+ are equalized. This makes it possible to maintain an ion balance between the external and inner medium on a lower metabolic basis than in neutral and slightly base media. In certain species of crustaceans and mollusks, calcium metabolism is more sensitive to a decline in the pH than is sodium metabolism.

INTRODUCTION AND DISCUSSION

Early experimental work dealing with the effect of active environmental reaction on fish established that O_2 uptake from water diminishes with decline of pH (Wiebe et al. 1934). The toxic effect was attributed to coagulation of gill mucus and membranes of gill epithelium causing "anoxia of coagulate film" (Ellis 1937). The more precise physiological effect of this film on the process of oxygen uptake from water was investigated relatively recently (Ultsch and Gros 19--). It was established that mucus secreted under the effect of acid provides only partial decrease in permeability of fish gills for oxygen. As a result, asphyxia develops very slowly and does not cause death.

---

[1] Institute of Biology of Inland Waters, USSR Academy of Sciences, Borok, USSR

A decline of medium pH leading to acidulation of blood elicits in most aquatic animals a decrease in affinity of respiratory pigments for oxygen. The dissociation curve shifts in the direction of higher PO_2 (Bohr effect) with decline of pH. This reflects the correlation between affinity of respiratory pigments for oxygen and their acid-base properties. On the basis of this information, as well as data concerning significant coagulation of mucus on fish gill epithelium, it was believed for a long time that the toxic effect of low pH is related to asphyxia. Subsequent studies refuted the role of asphyxia in acid poisoning as the primary cause of fish death (Vinogradov 1979, Eddy 1974). Acidulation of medium to a pH of 4.5 elicits a decrease in oxygen uptake in the carp, and it is restored within 24 h. After acclimation of fish to hypoxia, oxidation of water did not cause a decrease in oxygen uptake. The results of experiments showed that there is something in common in adaptation mechanisms that react to medium oxidation and hypoxia (Hoglund 1961, Vinogradov 1979). Respiratory systems adapt to ph decline primarily by means of increase in oxygen capacity of blood due to increase in hemoglobin, hematocrit, and erythrocyte content (Vaala and Mitchell 1970).

At the present time, it can be considered established that the toxic effect of low medium pH is based on functional impairment of systems of ionic and osmotic regulation localized in the gill epithelium of aquatic animals (Maetz 1974, McWilliams 1980, Packer and Dunson 1970, Packer and Dunson 1972, Spry et al. 1981, Vinogradov et al. 1976, Vinogradov et al. 1978). At the present time, it is assumed that specialized chloride cells that maintain a specific Na^+ level in blood are possibly the location of systems of ion transport against a concentration gradient in fish (Laurent and Dunel 1980). The main element of ionic regulation in freshwater animals is active transport of Na^+, K^+, Ca^{++} and Cl^- by the gills from water to the endogenous environment, as well as removal from the body of metabolites such as NH_4^+ H^+, HCO_3^- (Maetz 1973, Payan et al. 1981). Studies conducted recently furnished more information about the mechanism of regulation of acid-base equilibrium in fish and crustaceans. It was found that Na^+/H^+, Na^+/NH_4^+, HCO_3^-/Cl^- turnover in gills of freshwater fish is of substantial significance to maintaining blood ph (Cameron and Randall 1972, Cameron and Randall 1974, Eddy 1974, Randall and Cameron 1973, Renzis and Maetz 1973). The correlation between ion uptake through the gills and acid-base regulation was studied comprehensively by Haswell et al. (1980) and Perry et al. (1981). The rather low permeability of gill epithelium for water and salts plays an important part in adaptation to life in fresh water (Packer and Dunson 1970, Packer and Dunson 1972, Vinogradov et al. 1976, Vinogradov et al. 1978). Exogenous factors, which increase gill permeability, should cause excessive discharge of electrolytes and cause penetration into the body of undesirable substances.

Another characteristic that is very important to processes of ionic regulation in freshwater animals is affinity of ion-absorbing systems for concentration of substrate (Na^+, K^+, Ca^{++}, Cl^-) in the exogenous environment, which is usually referred to as the semisaturation constant (Harris 1972, Shaw 1959, Sutcliffe 1968, Sutcliffe 1971, Vinogradov 1978). In typical freshwater species, sodium absorption begins with a concentration of about 0.5 mg/ℓ, while saturation occurs at 5-10 mg/ℓ Na^+. The values are considerably lower for potassium ions. The kinetics of Ca^{++} absorption are similar to those for Na^+ ions. Ca^{++} absorption begins at concentrations of 0.4-0.5 mg/ℓ. This process reaches a maximum rate at 10-40 mg/ℓ, and the values for Na^+ and Ca^{++} ions are similar. The rate of K^+ absorption from water is 1/5th-1/10th the rate for Na^+ and Ca^{++} (Vinogradov et al. 1983, Vinogradov et al. 1984).

It was established in several studies that a decline of pH increases significantly loss of Na^+, K^+, Cl^- and Ca^{++} ions in fish, crustaceans and mollusks (Packer and Dunson 1970, Packer and Dunson 1972, Vinogradov et al. 1976, Vinogradov et al. 1979, Vinogradov et al. 1983). Oxidation of the medium causes manifold increase in overall Na^+ loss in fish (Figure 1a) and impairs Ca^{++} metabolism. Similar findings have been made in crustaceans and mollusks (Figure 1b). Threshold pH levels impairing gill permeability for ions are different in different animal species. In the perch (Perca fluviatilis L.), which is one of the most acid-resistant and common fish species, in reservoirs with high concentration of hydrogen ions, critical values of pH are considerably lower than for other species of fish. Investigation of rate of loss in perch inhabiting mildly alkaline water (Rybinskiy Reservoir, pH 7.6-8.0) and acid lakes (Lake Motykino and Lake Dubrovskoye in Volgograd Oblast, pH 3.5-4.2) revealed that permeability of gill epithelium for Na^+ is one-half less in lake fish than those in reservoirs (rivers).

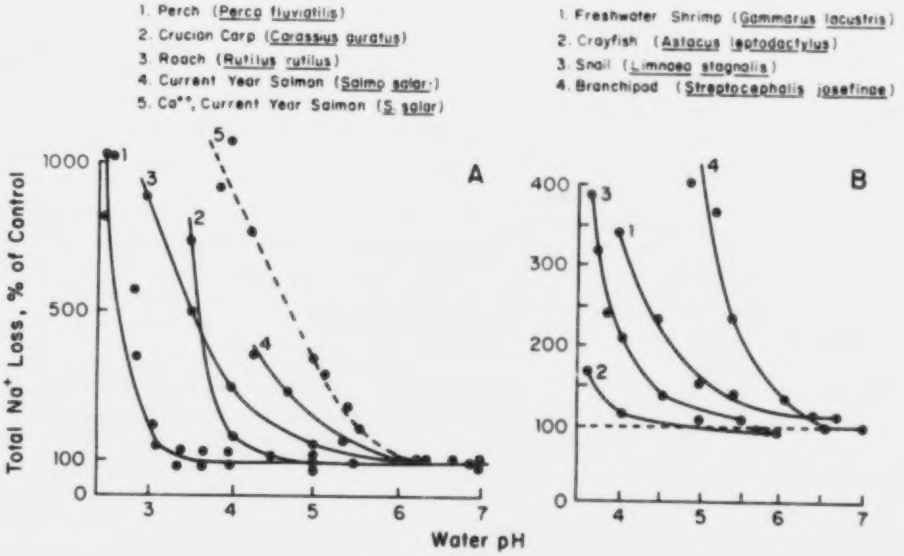


Figure 1. Effect of low pH on rate of total loss of Na^+ in fish(A) and invertebrates (B).

These differences in permeability become insignificant after perch from an alkaline environment undergo 6-day acclimation to water with pH 3.35 (Table 1). The threshold values for ambient pH eliciting drastic increase in gill permeability for ions, as well as resistance to oxidation, are the same in both groups of perch. These data indicate, in our opinion, the phenotypic nature of differences in fish from different bodies of water.

TABLE 1. EFFECT OF ACCLIMATION TO pH 3.35 ON Na^+ LOSS IN PERCH FROM LAKE AND RESERVOIR POPULATIONS

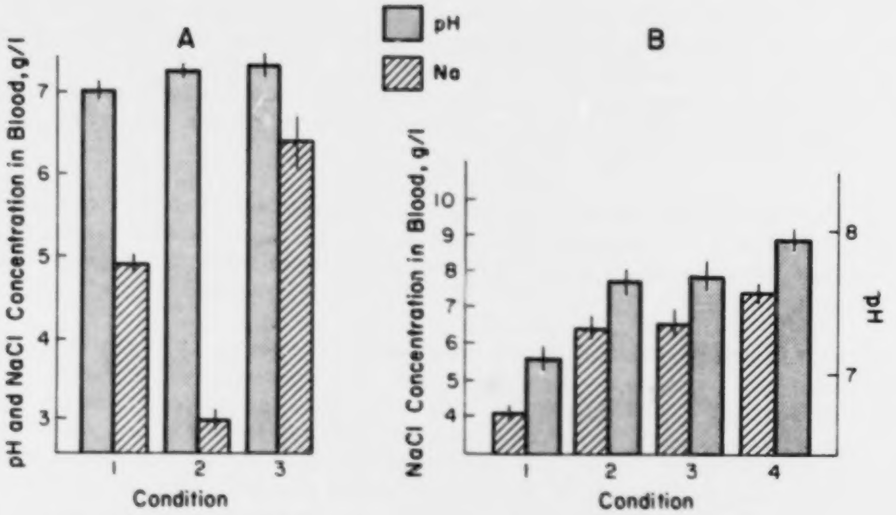
Acclimation, days	Na^+ loss, μmol/(g·h)	
	lake	reservoir
0	0.25±0.14	0.48±0.12
3	0.22±0.08	0.25±0.13
6	0.19±0.09	0.24±0.11

As shown by field and laboratory studies, decrease in sodium concentration of blood plasma and its oxidation result from increased permeability of gills for ions (Leivestad and Muniz 1976, Maetz 1973, Packer and Dunson 1970). Data in the literature indicate that decline of Na^+ content of blood and hemolymph of crustaceans is the chief cause of rapid death of fish and crustaceans in fresh water at low pH (Vinogradov et al. 1978, Vinogradov 1979, Vinogradov 1981, Vinogradov et al. 1983). Figure 2 and Table 2 provide data on change in concentration of Na^+ and pH in the endogenous environment.

Studies of survival revealed that freshwater shrimp (Gammaracanthus lacustris) tolerates greater oxidation of medium (pH 4.1-4.2) in diluted salt water than in fresh water (pH 5.0-5.1). Death of the shrimps in diluted salt and fresh water occurs at different hemolymph pH and different concentrations of ions.

In freshwater shrimp adapted to salt water, Na^+ transport through the gills is inactivated. Ion content of hemolymph is due to their levels in the external environment, and the cavitary fluid is isotonic in relation to the external environment. Hemolymph is hypertonic in fresh water, in relation to the external environment.

In diluted salt water, these animals die at hemolymph pH 6.3-6.4 and in fresh water at higher pH. Ion content of hemolymph in shrimp from fresh water diminishes with oxidation of water and reaches the same critical values before death as in desalinated distilled water. The toxic effect of low pH is drastically enhanced when Na^+ concentration in water is less than 0.6-0.7 mmol/ℓ. Gammaracanthus adapted to water with 0.4 mmol/ NaCl died at pH 5.5-5.7. This is probably related to diminished absorption of Na^+ from water containing less than 0.6 mmol/ℓ, as well as decrease in sodium concentration and osmotic pressure of hemolymph due to



(1-distilled water, pH 3.7;
2-distilled water, pH 5.6-6-5;
3-fresh water, pH 7.5)

(1-distilled water, pH 3.8;
2-river water, pH 3.8;
3-distilled water plus 70mg/lCa^{++},
4-river water, pH 7.4)

Figure 2. pH and Na$^+$ concentration in blood of roach (*Rutilus rutilus*) (A) and crucian carp (*Carassius auratus*) (B) as a function of different ambient conditions, according to Vinogradov et al., 1983, Vinogradov et al., 1983, Vinogradov et al., 1978.

TABLE 2. CONCENTRATION OF ELECTROLYTES AND pH OF HEMOLYMPH BEFORE DEATH OF *GAMMARACANTHUS LACUSTRIS* IN ACID MEDIUM AND DISTILLED WATER

pH	Acclimation environment	Electrolyte and Na$^+$ concentration in hemolymph, μmol (scaled to NaCl) electrolytes	sodium	Hemolymph pH	Number of experimental animals
3.1	Diluted salt water (12%)	310 ± 15	255 ± 5	5.35 ± 0.1	5
3.5	Fresh water	105 ± 10	100 ± 10	6.6 ± 0.2	4
3.5	Diluted salt water (12%)	305 ± 15	255 ± 10	6.4 ± 0.1	5
4.5	Fresh water	105 ± 15	90 ± 15	6.95 ± 0.15	6
7.0	Distilled water	--	90 ± 5	7.6 ± 0.1	6

insufficient affinity of the sodium-transporting system in this range of concentrations. Among crustaceans, as in fish, acid-resistant species encountered in acid waters do not present excessive loss of salts in experiments over a wide range of changes in concentration of hydrogen ions. According to our data, threshold pH increasing discharge of Na^+ in the hog slater (*Asellus aquaticus*) is 3.1-3.2 and for the freshwater shrimp (*Cammarus lacustris*) 5.5-5.6, which is consistent with the results of field studies (Malley 1980).

Thus, the viability of some species of freshwater fish and crustaceans in acid waters is largely determined by the tolerance of integument, primarily gill epithelium, to low ambient pH without change in permeability of these tissues for monovalent Na^+, Cl^- and H^+ ions.

General tissue permeability to substances is determined by its cellular (membrane) and intercellular elements. The correlation between rates of excessive loss of Na, K^+ and Cl^- ions through fish gills in an acid medium corresponds to the concentrations of these ions in blood and their mobility (Vinogradov et al. 1979). This is indicative primarily of increase in intercellular permeability in an acid medium. Electron microscope studies of reaction of fish gill epithelium to an acidulated medium revealed that the increase in passive exit of Na^+ under the effect of low pH is attributable to impairment of intercellular interactions in gill epithelium. Thus, in the crucian carp placed in water at pH 3.6, there is impairment of integrity of external cell membranes and lysis of some parts of them (Vinogradov et al. 1983). There is widening of intercellular spaces in the region of simple connections. In some cases, there is partial impairment of solid connections of the apical segments of gill epithelial cells.

On the basis of data concerning excessive loss of Na^+, K^+ and Cl^- in an acid medium, as well as the capacity of calcium ions to normalize these losses, the hypothesis was expounded that the protective effect of Ca^{++} on fish at low pH is based on the properties of Ca^{++} to cement intercellular contacts with exposure to factors that disintegrate the gill epithelium (Vinogradov et al. 1979). With pH 3.8, Ca^{++} normalizes not only Na^+ metabolism in *Carassius carassius* L., but maintains acid-base homeostasis. In the absence of Ca^{++} ions, there is a drastic drop of blood Na^+ level. It is oxidized (Figure 2) and fish death occurs within 7-8 h. Ca^{++} capacity to stabilize in an acid medium both sodium and acid-base homeostasis indicates that Ca^{++} ions limit gill permeability not only to Na^+, but to H^+.

It was established in several works that Ca^{++} and Mg^{++} ions affect permeability of gill epithelium for Na^+ and Cl^- in freshwater and euryhaline fish (Evans 1975, Maetz 1974, Potts and Fleming 19--). In our experiments, we studied the effect of Ca^{++} on permeability of gill epithelium of the crucian carp (*Carassius auratus*) for Na^+ in a neutral (pH 6.8-7.0) and an acid medium (pH 3.8). It was shown that, with a neutral medium reaction, the increase in Ca^{++} concentration in water lowers the discharge of Na^+ from the body. An increase in Ca^{++} content beyond 30 mg/ℓ has virtually no effect on loss of Na^+ through the gills, whereas a decrease in

Ca^{++} concentration to less than 10 mg/ℓ elicits drastic increase in permeability of gills in the crucian carp. A drop of pH from 6.8 to 3.8 in distilled water increases by many times the rate of loss of Na$^+$ in fish (Figure 3a). These data indicate that an increase in Ca^{++} concentration in water has an effect on gill epithelium that is the opposite of that of decline of pH of the medium.

The main changes in gill permeability for Na$^+$ are observed within the first 15 min after addition of Ca^{++} to both an acid (pH 3.8) and neutral (pH 6.8) medium (Figure 3b). However, Na$^+$ loss decreases to less than 1/5th in acidulated distilled water with added Ca^{++}, as compared to a neutral medium. The dynamics of the process of increase in permeability in crucian carp placed in distilled water at pH 3.8 are inversely proportionate to the change under the effect of Ca^{++}. The most significant increase in permeability is observed within the first few minutes of exposure of the fish to an acid medium (Figure 3b).

Investigation of total Na$^+$ loss by fish as related to change in ion composition of water established that the decline in yield of Na$^+$ with decline of water pH is not a specific reaction. A decrease in total Na$^+$ loss is observed in the perch (Perca fluviatilis) and salmon fry (Salmo salar) when kept for a long time in distilled water and $CaCl_2$ solution (Figure 4). Analysis of the results enables us to conclude that insufficient intake of Na$^+$, regardless of causes, leads to decrease in Na$^+$

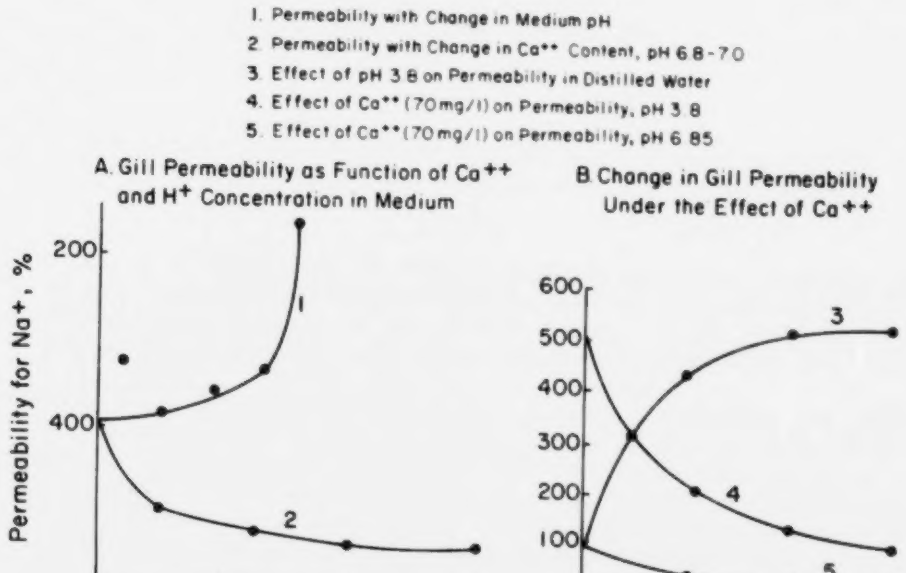


Figure 3. Effect of external concentration of Ca^{++} and H$^+$ on permeability for Na$^+$ of crucian carp gills according to Vinogradov, 1983.

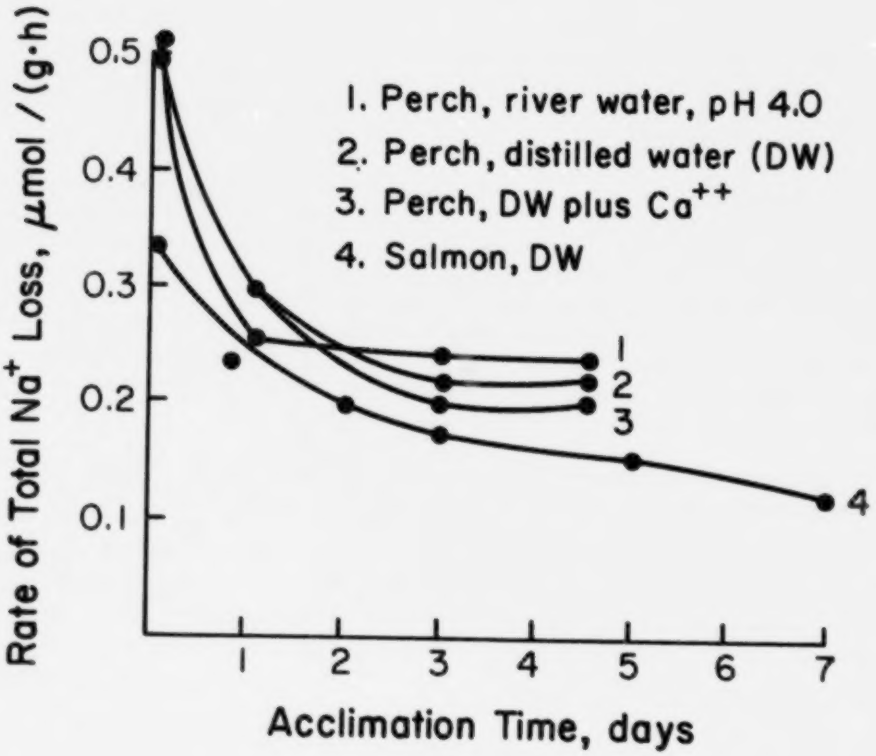


Figure 4. Effect of desalination and low pH on Na^+ loss in the perch (Perca fluviatilis) and current year salmon (Salmo salar).

loss and, probably, decrease in permeability of gill epithelium. Evidently, Ca^{++} ions are not of basic significance to regulation of gill permeability for Na^+ under natural conditions at optimum pH values in freshwater fish capable of living in waters with low mineral content.

Unlike the phenomenon of increased gill permeability, which is distinctly manifested only with lethal or close to lethal pH values, inhibition of Na^+ transport through the gills of freshwater fish is observed in the tolerated range of pH values (Maetz 1973, Maetz 1974, Packer and Dunson 1970, Packer and Dunson 1972). The extent of inhibition of Na^+ absorption of fish depends on ambient pH (Figure 5). However, the data obtained in short-term experiments reflect only the initial stage of reactions of sodium- and chlorine-transport systems of freshwater fish to oxidation of medium. Studies dealing with acclimation of fish to decline of medium pH revealed that there is partial or complete restoration of Na^+ absorption function during acclimation, depending on intensity of exposure and fish species (Sokolov and Vinogradov 1977). The capacity of the sodium-transport system of fish to adapt to acidulation of medium was confirmed in subsequent works (McWilliams 1980, Thurston 1979a, Vinogradov et al. 1979). For example, with acclimation of the crucian carp to a pH of 5.5, the rate of Na^+ absorption was significantly slower already within 3-6 h than in control fish (52-40%). Na^+ sorption increased 12 h after the start of the experiment, and it constituted 70% and 78% of initial level after 24 and 48 h, respectively (Figure 6). A decline of pH to 4.5 after 48-h acclimation of the crucian carp to a pH of 5.5 again elicited a decrease in Na^+ transport. Na^+ absorption after 7 days of acclimation constituted only 50% of the Na^+ sorption level in control fish (Thurston et al. 1979a).

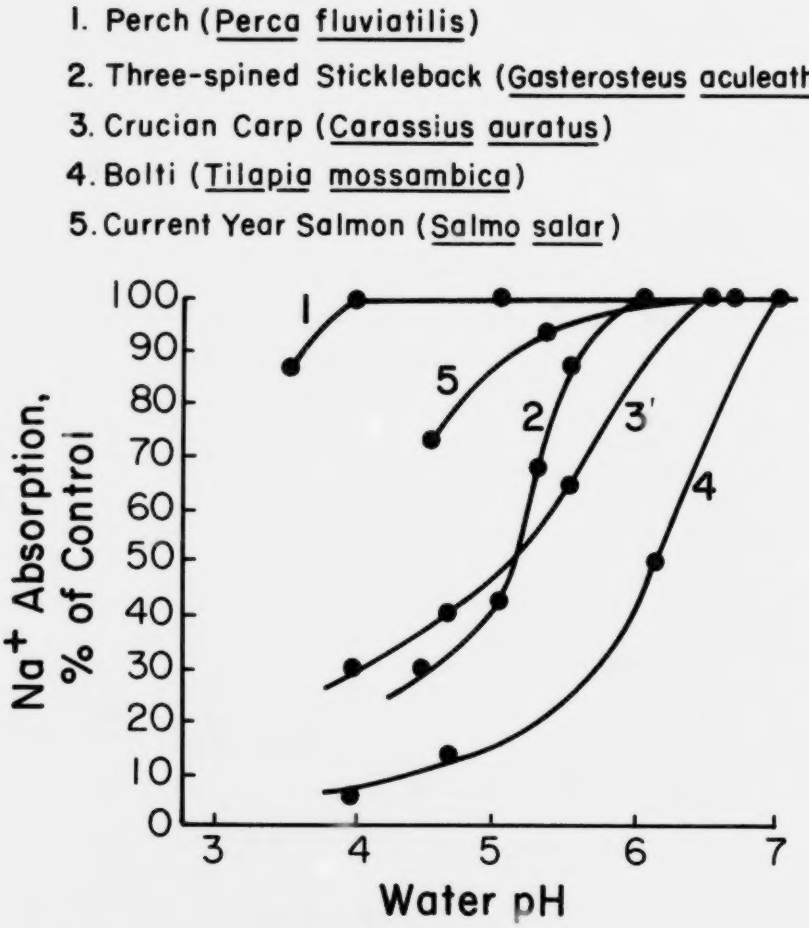


Figure 5. Na^+ absorption in fish as a function of ambient pH, according to Vinogradov et al., 1979.

Decrease in activity of different enzymes could be a probable cause of depression of Na^+ and Cl^- transport in fish gills with change in ambient pH. This applies, first of all, to Na^+, K^+, Mg^{++}-activated ATPases and succinate dehydrogenase (SDH), which are enzymes directly involved in transmembrane ion transport. At the present time, it is assumed that active Na^+ transport through cell membranes is affected by the function of Na^+, K^+-ATPase, which is a good explanation for the presence of ionic gradients between intracellular and extracellular media.

Na^+, K^+-ATPase, however, constitutes an insignificant part of overall ATPase activity of gill tissue to maintain ionic assymetry between cells of the gill epithelium in fresh water, which has very low levels of ions (Thurston et al. 1979b). Strophanthin-resistant ATPase plays the leading role (over 90% of all ATPase activity). The optimum pH for Na^+, K^+-ATPase and strophanthin-resistant ATPase is in the range of 7.5 to 8.5, and it does not change when fish are acclimated to a more acid medium. Na^+, K^+-ATPase activity remains stable during 10 days of acclimation of the carp to a pH of 5.5. The strophanthin-resistant component of overall ATPase activity increases during acclimation to low pH. Reliable differences were noted one day after the start of the experiment (Figure 7).

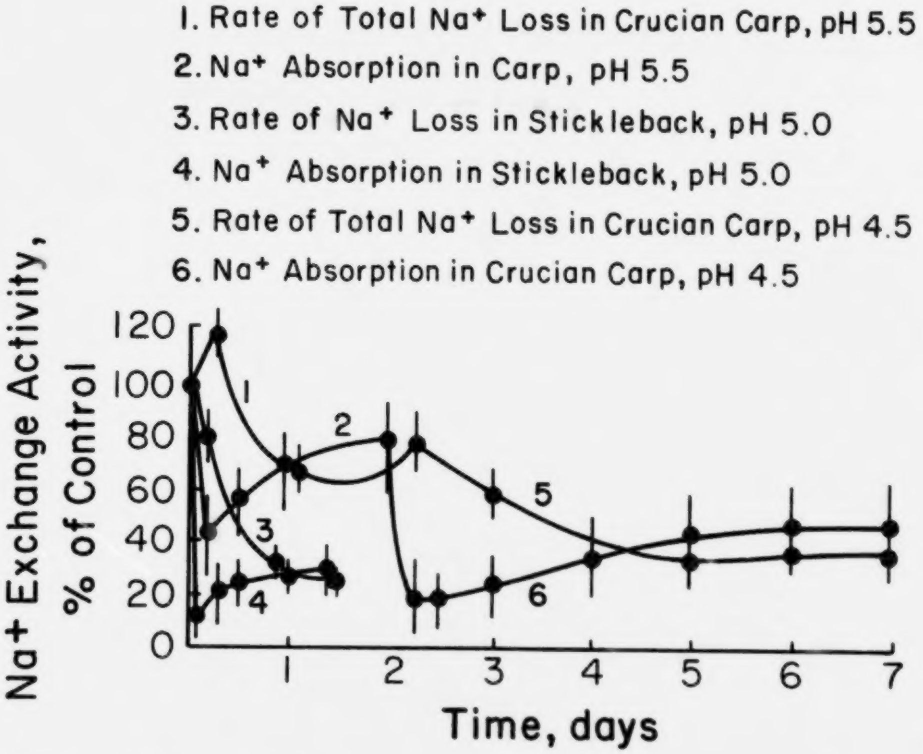


Figure 6. Effect of low pH on Na^+ exchange in crucian carp (*Carassius auratus*, according to Thurston et al., 1979, and stickelback (*Gasterosteus aculeathus*), according to Matey et al., 1981.

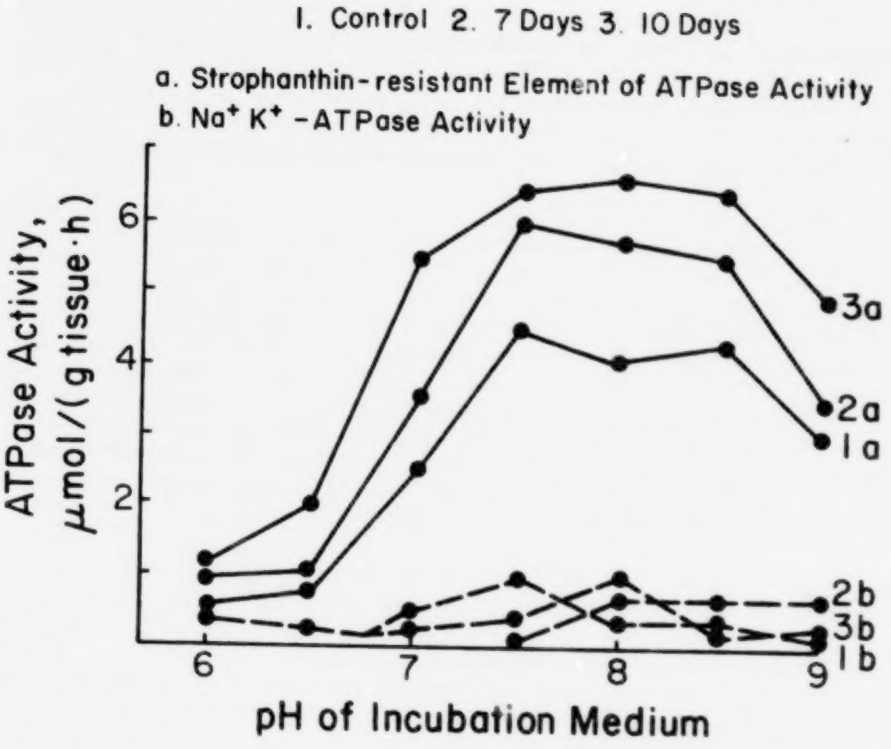


Figure 7. ATPase activity in crucian carp (Carassius auratus) gills with acclimation to pH 5.5, according to Thurston et al., 1979.

These results warrant the belief that, in freshwater fish, strophanthin (ouabain)-insensitive ATPase plays an important part in ion regulation.

The results indicate that there are two concurrent adaptive processes in fish during acclimation to low pH. On the one hand, there is less loss of Na$^+$ from the body and, on the other hand, after an initial significant inhibition of Na$^+$ absorption, there is partial restoration of rate of sorption of this ion, which makes it possible to maintain a balance between external and internal Na$^+$.

In the crayfish, Astacus leptodactylus, Na$^+$ exchange in gills, like in the hog slater, Asellus aquaticus, is not sensitive to pH drop in the range of 6.0-4.0. However, acidulation of the environment elicits a drastic increase in loss of Ca^{++}. In crayfish placed in water with pH of 4.5, the rate of general loss of Ca^{++} increases by 8-9 times, as compared to the control, and remains unchanged for 7 days. Threshold pH at which Ca^{++} absorption does not compensate for its loss is 4.6-4.7 (Figure 8a). At neutral pH, Ca^{++} sorption in crayfish is 7-8 times greater than loss (Vinogradov et al. 1983). This creates conditions for accumulation and deposition in the body of Ca^{++} required for crayfish growth and ecdysis.

Apparently, impairment of Ca^{++} exchange, along with changes in regulation of Na^{+} and Ca^{++} uptake when the environment was acidulated (Figure 8a and b). Investigation of Ca^{++} and Na^{+} metabolism revealed that there is increase in total loss of Ca^{++} and Na^{+} in mollusks starting at a pH of less than 6.5. In our opinion, impairment of Ca^{++} metabolism is of primary significance to survival of mollusks, since the intensity of their uptake of Ca^{++} is substantially greater than its uptake by fish and crustaceans (Figure 9).

Investigation of electric conductivity of distilled water titrated with HCl and H_2SO_4 to different pH values after placing freshwater shrimp, Gammaracanthus lacustris, in it revealed that total electrolyte content of water at pH of less than 4.3 diminishes drastically, in spite of the significant migration into water of Na^{+} and Cl^{-} ions from the body (Figure 10). The results of this experiment are indicative of considerably greater change in gill permeability for hydrogen ions than for salts at low environmental pH. Analogous data, which revealed that permeability of the integument of crustaceans for H^{+} in an acidulated environment is much greater than for other ions and that intake of H^{+} exceeds total output of salts, were also obtained for other crustacean species.

On the basis of many years of our own studies and analysis of data in the literature, it was determined that fish and invertebrate death with decline of pH was due essentially to impairment of processes of ionic regulation. In an acid environment, exchange of Na^{+}, Cl^{-} and Ca^{++} between the organism and water shifts in the direction of excessive escape of these ions into the environment, and there is unbalanced intake of H^{+}.

Low, acutely toxic pH levels increase permeability of fish gill epithelium for ions. In this case, the rate of diffusion of salts from the body increases by several times. Blood electrolyte concentration de-

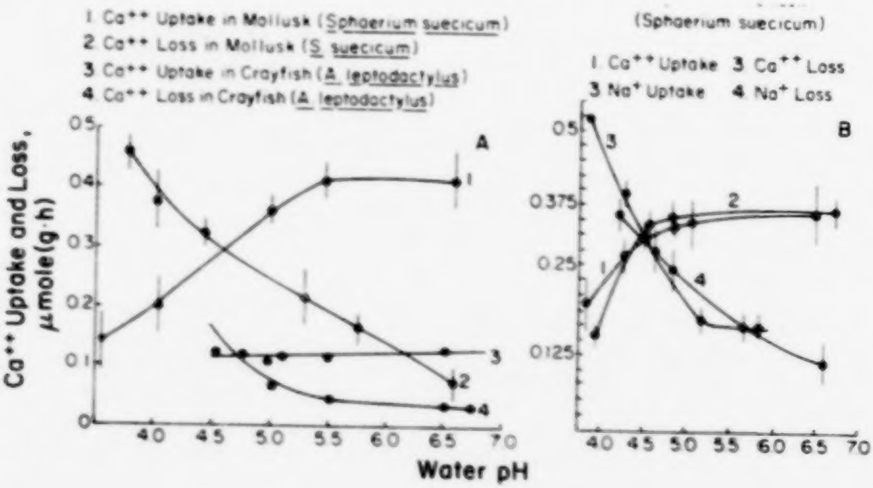


Figure 8. Uptake and loss of ions at different environmental pH in invertebrates.

creases rapidly, there is intensive passage of hydrogen ions over concentration gradient to the endogenous environment, and concurrently with desalination there is development of acidosis. At such pH values, there are serious destructive changes in the gill epithelium, which are the most intensive in a medium with less than 20-40 mg/ℓ Ca^{++}.

Low sensitivity of transepithelial Na^+ transport and gill permeability for ions with drop of ambient pH are typical features of one of the most acid-resistant fish species, the perch. In an acid medium, Ca^{++} ions normalize both sodium and acid-base homeostasis in fish thanks to the capacity of this cation to limit gill permeability for Na^+ and H^+ by means of "cementing" intercellular contacts and stabilizing external cytoplasmic membranes.

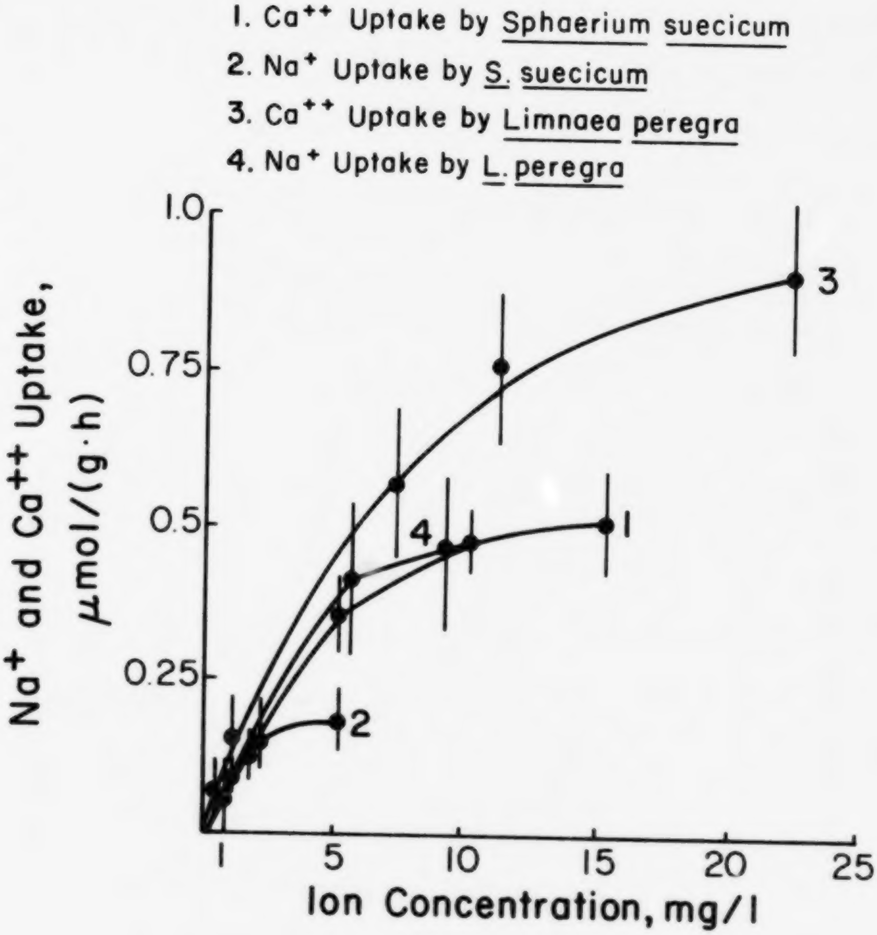


Figure 9. Ca^{++} and Na^+ uptake in freshwater mollusks Limnaea peregra and Sphaerium suecicicum as a function of concentration of these ions in water.

The pH values that elicit substantial changes in permeability of gill epithelium for Na^+ correspond to a low range of pH, at which adult specimens of different species are encountered under natural conditions.

In the tolerated range of low pH, fish adaptation occurs due to phasic changes in activity of metabolic processes and alteration of structural organization of gill epithelial cells. A decline of ambient pH initially depresses Na^+ uptake, stimulation of synthetic activity of cells, and SDH activity in gill epithelium. Subsequently, development of these processes slows down and at the next stage there is partial restoration of Na^+ transport, decrease in permeability of gill epithelium, stablization of metabolism, but usually at a lower level than in a neutral and mildly alkaline environment.

Strophanthin-resistant ATPase is apparently very important to osmotic regulation in freshwater fish. Activity of Na^+, K^+-ATPase constitutes an insignificant part of overall ATPase activity of gills. The role of Na^+, K^+-ATPase in gills of freshwater fish is apparently limited to regulation of intracellular proportions of Na^+ and K^+.

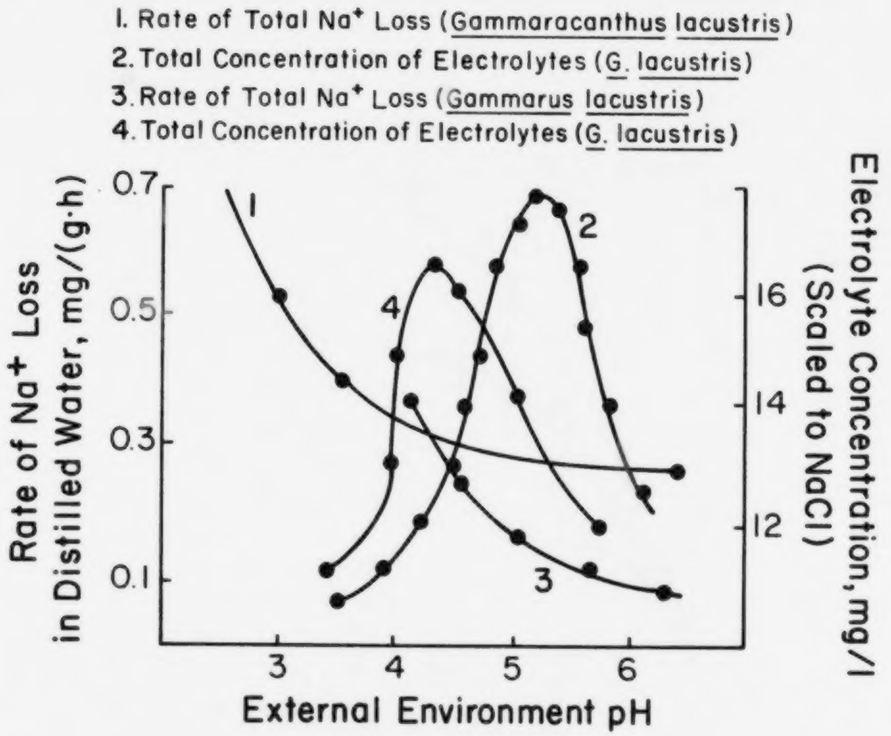


Figure 10. Rate of total loss of Na^+ and electrolyte concentration in distilled water titrated with HCl as a function of environmental pH in freshwater shrimp.

There is impairment of processes of ion regulation in the case of acidulation, in crustaceans and mollusks, as well as fish. One observes increased loss of Na^+ and Ca^{++}, and inhibition of Na^+ uptake from the environment. Calcium metabolism is more sensitive to a decline of pH than sodium metabolism in some species of crustaceans and mollusks. This warrants the belief that the changes in regulation of Ca^{++} that occur in an acid medium could restrict the existence of some species in waters with low environmental pH.

BIBLIOGRAPHY

Cameron, J.N. and D.R. Randall. 1972. The effect of increased ambient CO_2 on arterial CO_2 tension, CO_2 content and pH in rainbow trout. J. Exp. Biol. 57(3).673-680.

Cameron, J.N. and F.A. Polhemus. 1974. Theory of CO_2 exchange in trout gills. J. Exp. Biol. 60(1):183-194.

Eddy, F.B. 1974. Acid-base balance in rainbow trout (*Salmo Gairdneri*) subjected to acid stresses. J. Exp. Biol. 64(1):159-171.

Ehrenfeld, J. 1974. Aspects of ionic transport mechanisms in crayfish *Astacus Leptodactilus*. J. Exp. Biol. 61(1):57-70.

Ellis, M.M. 1973. Detection and measurement of stream pollution. Bull. Bur. Fisheries. 48(22):365-437.

Evans, D.H. 1975. Ionic exchange mechanisms in fish gills. Comp. Biochem. Physiol. 51(491-495).

Harris, R.R. 1972. Aspects of sodium regulation in a brackish water and a marine species of the isopod genus sphaeroma. 12(1):8-27.

Haswell, M.S., D.J. Randall, and S.F. Perry. 1980. Fish gill carbonic anhydrase. acid-base regulation or salt transport? Am. J. Physiol., 238.240-268.

Hoglund, L.B. 1961. The reaction of fish in concentration gradients. Rep. Inst. Freshwater Res., 43:1-147.

Kerstetter, T.H., and L.B. Kirschner. 1972. Active chloride transport by the gills of rainbow trout (*Salmo Gairdneri*). J. Exp. Biol. 56(1). 263-272.

Laurent, P. and S. Dunel. 1980. Morphology of gill epithelia in fish. Amer. J. Physiology. 238(3):147-159.

Leivestad, H. and I. Muniz. 1976. Fish kill at low ph in a Norwegian river. Nature (London). 259:391-392.

Maetz, J. 1972. Branchial sodium exchange and ammonia excretion in the goldfish *Carassius Auratus*. Effects of ammonia-loading and temperature changes. J. Exp. Biol. 56.601-610.

Maetz, J. 1973. Na^+/NH_4^+, Na^+/H^+ exchanges, NH_3 movement across gill of *Carassius Auratus*. J. Exp. Biol. 58(1):255-275.

Maetz, J. 1974. Origin of difference in transbranchial electric potential in *Carassius Auratus* goldfish. Importance of Ca^{++} ion. C.R. Acad. Sci. (Paris). 279:1277-1280.

Maetz, J. and F. Garcia-Romeu. 1964. The mechanism of sodium and chloride uptake by the gills of a freshwater fish *Carassius Auratus*. 2: Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. J. General Physiology. 47(7):1029-1229.

Malley, D. 1980. Decreased survival and calcium uptake by the crayfish *Orconectes Viridis* in low pH. Can. J. Fish. Aquatic Sciences. 37.364-372.

Matey, V.Ye., A.D. Kharazova, and G.A. Vinogradov. 1981. Reaction of chloride cells of gill epithelium of stickleback, *Gasterosteus Aculeatus L.*, to change in pH and salinity of environment. Tsitologiya. 23(2).159-165. pp. 159-165.

McWilliams, P. 1980. Effect of ph on sodium uptake in Norwegian brown trout (*Salmo Trutta*) from an acid river. J. Exp. Biol. 88:259-267.

Packer, R.K. and W.A. Dunson. 1970. Effects of low environmental pH on blood pH and sodium balance of brook trout. J. Exp. Zool. 74(1). 65-72.

Packer, R.K. and W.H. Dunson. 1972. Anoxia and sodium loss associated with the death of brook trout at low pH. Comp. Biochem. Physiol. 41A.17-26.

Payan, P., N. Mayer-Gostan, and P. Pang. 1981. Site of calcium uptake in ionic freshwater trout gill. J. Exp. Zool. 216:345-347.

Perry, S.F., M.S. Haswell, D.J. Randall, and A.P. Farrell. 1981. Branchial ionic uptake and acid-base regulation in the'rainbow trout *Salmo Gairdneri*. J. Exp. Biol. 92:289-303.

Potts, W.T.W. and W. Fleming. (No year given). The effect of prolaction and divalent ions on the permeability to water of the Fundulus Kansae, J. Exp. Biol. 53:317-327.

Randall, D.J. and J.N. Cameron. 1973. Respiratory control of arterial pH as temperature changes in rainbow trout Salmo Gairdneri. Amer. J. Physiol. 225(5).997-1002.

Renzis, G. and J. Maetz. 1973. Studies on the mechanisms of chloride absorption by the goldfish gill, relation with acid-base regulation. J. Exp. Biol. 59(2).339-358.

Shaw, J. 1959. The absorption of sodium ions in the crayfish Astacus *Pallipes Lereboullet*. 1: The effect of external and internal sodium concentrations. J. Exp. Biol. 36(1):126-144.

Sokolov, V.A. and G.A. Vinogradov. 1977. Investigation of fish adaptation to different environmental pH. In. Biologiya vnutrennikh vod. Inform. byul. (Biology of Inland Waters. Information Bulletin). No. 33. Leningrad, USSR. pp. 56-59.

Spry, D., C. Wood, C. and P. Hodson. 1981. The effects of environmental acid on freshwater fish with particular reference to the soft water lakes in Ontario and the modifying effects of heavy metals. A literature review. In: Canadian Technical Report of Fisheries and Aquatic Sciences. Toronto, Canada. 999 pages.

Sutcliffe, D.W. 1968. Sodium regulation and adaptation to fresh water in Gammarid crustaceans. J. Exp. Biol. 48:335-380.

Sutcliffe, D.W. 1971. Sodium influx and loss in freshwater and brackish-water population of the amphipod Gammarus Duebeni Lilljebirg. J. Exp. Biol. 54(1).255-268.

Thurston, R.V., G.A. Vinogradov, V.T. Komov, and V.Yu. Matey. 1979. Effect of low pH, ammonia salts and desalination on enzyme activity, sodium metabolism in gills and ultrastructure of chloride cells in freshwater fish. Report 1. In: Biologiya vnutrennikh vod. Inform. byul. pp. 75-81.

Thurston, R.V., G.A. Vinogradov, V.T. Komov, and V.Ye. Matey. 1979b. Effect of low pH, ammonia salts and desalination on enzyme activity, sodium metabolism in gills and ultrastructure of chloride cells in freshwater fish. Report 2. In: Biologiya vnutrennikh vod. Inform. byul. No. 44. pp. 75-79.

Ultsch, G. and G. Gros (No year). Mucus as a diffusion barrier to oxygen: Possible role in O_2 uptake at pH in carp (Cyprinus Caprio) gills. Comp. Biochem. Physiol. 62A:685-689.

Vaala, S. and R. Mitchell. 1970. Blood oxygen tension changes in acid exposed brook trout. Proceed. Pennsylv. Acad. Sci. 44:41-44.

Vinogradov, G.A. 1976. Osmotic regulation of some relict glacier crustaceans as related to distinctions of their ecology and origin. In Solenostnyye adaptatsii vodnykh organizmov (Salt Adaptation of Aquatic Organisms). Leningrad, USSR. pp. 176-209.

Vinogradov, G.A. 1979. Adaptation of aquatic animals with different types of osmotic regulation to decline of environmental pH. In. Fiziologiya i parazitologiya presnovodnykh zhivotnykh (Physiology and Parasitology of Freshwater Animals). Leningrad, USSR. pp. 17-25.

Vinogradov, G.A. 1981. Processes of ionic regulation in freshwater animals in waters with anthropogenic pollution. In Biologiya vnutrennikh vod (Biology of Inland Waters). No. 51. Leningrad, USSR. pp. 53-56.

Vinogradov, G.A., P.A. Gdovskiy, and V.Ye Matey. 1979. Oxidation of water reservoirs and its effects on metabolism of freshwater animals. In: Fiziologiya i parazitologiya presnovodnykh zhivotnykh. Leningrad, USSR. pp. 98-116.

Vinogradov, G.A., Ye.S. Dal', and V.T. Komov. 1983. Investigation of basic gill functions of crayfish exposed to ammonia salts and acidulation of environment. In. Reaktsiya gidrobiontov na zagryazneniye (Hydrobiont Reaction to Pollution). Moscow, USSR. pp. 168-175.

Vinogradov, G.A., V.T. Komov, and V.Ye. Matey, 1984. Functional bases of effect of low ph on fish and crustaceans. In: Fiziologicheskiye i biokhimicheskiye aspekty toksikologii presnovodnykh zhivotnykh" (Physiological and Biochemical Aspects of Toxicology of Freshwater Animals) Manuscript filed with VINITI (All-Union Institute of Scientific and Technical Information), IBVV (Institute of Biology of Inland Waters), USSR Academy of Sciences, on 25 March 1984, No. 1637-84. pp. 147-190.

Vinogradov, G.A., V.Ye. Matey, V.Ye. and Ye.S. Dal'. 1983. Effect of calcium on mineral metabolism and gill ultrastructure in freshwater fish. In. Reaktsiya gidrobiontov na zagryazneniye. Moscow, USSR. pp. 199-207.

Vinogradov, G.A., V.A. Sokolov, and G.I. Fierova. 1976. Investigation of mechanism of action of low pH on freshwater fish. In. Teoreticheskiye aspekty rybokhozyaystvennykh issledovaniy vodokhranilishch (Theoretical Aspects of Fishery Studies of Reservoirs). Leningrad, USSR. pp. 168-173.

Vinogradov, G.A., V.A. Sokolov, and G.I. Flerova. 1976. Loss of sodium ion and changes in blood pH of fish exposed to acid environment. In: Tret'ya Vsesoyuznaya konferentsiya po ekologicheskoy fiziologii ryb. Tezisy dokl (Summaries of Papers Delivered at 3d All-Union Conference on Ecological Physiology of Fish) Part I. Kiev, USSR. pp. 76-77.

Wiebe, A., A. McGavock, A. Fuller, and H. Markus. 1934. The ability of freshwater fish to extract oxygen at different hydrogen ion concentrations. Physiol. Zool. 7:435-448.

AMMONIA PRODUCTION AND EXCRETION BY FISH

by
D.J. Randall[1]
P.A. Wright

ABSTRACT

Ammonia is continually produced and excreted by fish. High environmental ammonia levels, however, will reduce excretion resulting in body accumulations. In this paper, published research on the production, utilization, distribution, and excretion of ammonia by fish is reviewed.

INTRODUCTION

Ammonia is an end product of protein metabolism and, if allowed to accumulate in the body, has a toxic action. Ammonia must, therefore, either be excreted or be converted to less toxic compounds such as urea or glutamine. Ammonia is a substrate as well as a product of protein metabolism and in some tissues it may be utilized rather than produced. In general, however, there is a continual production and excretion of ammonia, or the less toxic substance urea, by the whole animal. Elevated environmental ammonia levels will reduce excretion and result in ammonia accumulation in the body of fish.

Although ammonia is almost completely ionized at body pH values, the gill is much more permeable to unionized ammonia and it appears that passive diffusion of NH_3 is the major form of ammonia excretion across the gills of freshwater fish. Ammonium ions may also be excreted in exchange for Na^+ ions. The pH gradients across the gills will influence ammonia excretion, which will vary according to blood and environmental water pH changes.

AMMONIA PRODUCTION AND UTILIZATION

Amino acids in excess of those required for protein synthesis are converted to ammonia in the liver. Figure 1 illustrates the sources and fate of ammonia in fish. Transaminases in the liver convert amino acids into glutamate for subsequent conversion into ammonia (Forster and Goldstein, 1969; Watts and Watts, 1974). Ammonia is also produced by the deamination of adenylates in fish muscle (Driedzic and Hochachka, 1976). Enzymes involved in ammonia production have been located in the kidney, gill, and muscle, as well as in the liver; however, the major site of ammonia production is probably the liver.

Ammonia toxicity can be ameliorated by the formation of less toxic compounds, namely glutamine and urea. Levi et al. (1974) recorded high levels of glutamine in the brain of goldfish and found that brain glutamine levels increased with ambient ammonia concentrations. Webb and Brown (1976) found high glutamine synthetase activity in the teleost and elasmobranch brain and this may be important in protecting the brain from sudden surges in ammonia concentration. Walton and Cowey (1977) were able to detect glutaminase activity in the gills of trout, but were unable to measure any *in vivo* utilization of glutamine by the gills.

---

[1] Dept. of Zoology, University of British Columbia, Vancouver, B.C. Canada

Ammonia can be converted, through carbamyl phosphate, to urea either via purines (uricolysis) or via the ornithine cycle. The enzymes required for uricolysis have been found in most fishes (Forster and Goldstein, 1969; Watts and Watts, 1974) but Florkin and Duchateau (1943) were unable to detect any activity of uricolytic enzymes in the cyclostome, *Lampetra*. The ratio of urea production via the ornithine cycle and uricolysis is about 100 to 1 in elasmobranchs and dipnoi, whereas in teleosts, most of the urea is formed via uricolysis (Gregory, 1977).

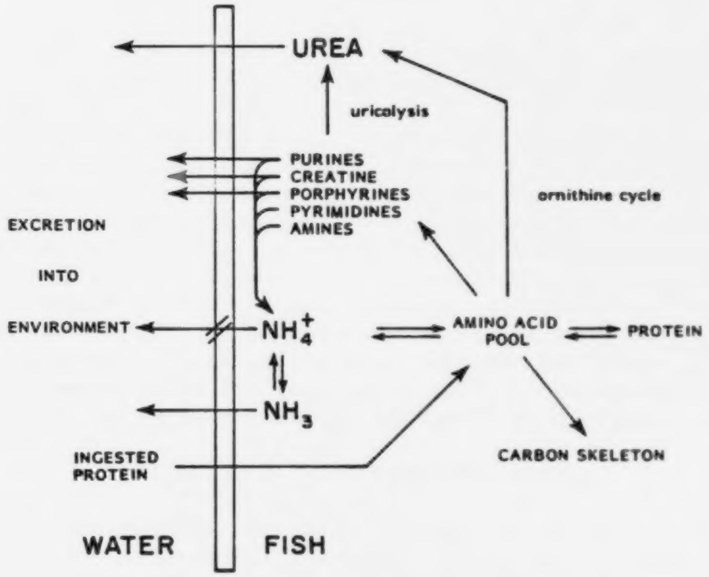

UREA
uricolysis
PURINES
CREATINE
PORPHYRINES
PYRIMIDINES
AMINES
ornithine cycle
EXCRETION
INTO
ENVIRONMENT
NH_4^+
AMINO ACID POOL
PROTEIN
NH_3
INGESTED PROTEIN
CARBON SKELETON
WATER
FISH

Figure 1. Production and excretion of nitrogenous compounds in fish.

AMMONIA DISTRIBUTION

Ammonia exists in aqueous solution either as ammonia gas or as ammonium ion. Trussell (1972) and Thurston et al. (1979) present tables of the percent unionized ammonia in solutions of different pH and temperature; the percent unionized ammonia increases with increasing pH and temperature but decreases with increases in ionic strength of the solution. The combined concentrations of ammonia gas in solution (NH_3) and ammonium ions (NH_4^+) will be referred to as total ammonia.

Cameron and Heisler (1983) found that ammonia was slightly more soluble in fish plasma than in water, and they also constructed a nomogram to describe the effects of ionic strength and temperature on the pK of the ammonia/ammonium reaction (see also Kormanik and Cameron, 1981; Boutilier et al. 1984). The pK is around 9.5, so at the pH of fish tissues, nearly all of the ammonia will be as ammonium ion. The pH of the tissue will be an important determinant of the total ammonia level. Ammonia gas diffuses at about the same rate as CO_2 (Cameron and Heisler, 1983) so it will rapidly equilibrate between different tissue compartments. Ammonium ion is not so permeable and large differences in levels may occur between compartments; in mammals and birds it has been shown that ammonium ion levels reflect the pH of the compartment, tissues with a lower pH having higher concentrations. This situation probably holds true for fish (Figure 2). Thus, the heart of a fish may have a much higher total ammonia concentration because the pH of this tissue is lower than that of the blood; this is due to elevated ammonium ion concentrations in heart muscle, with the ammonia gas levels being in equilibrium with the blood. In general very little is known about the difference in total ammonia content in different tissues in fish.

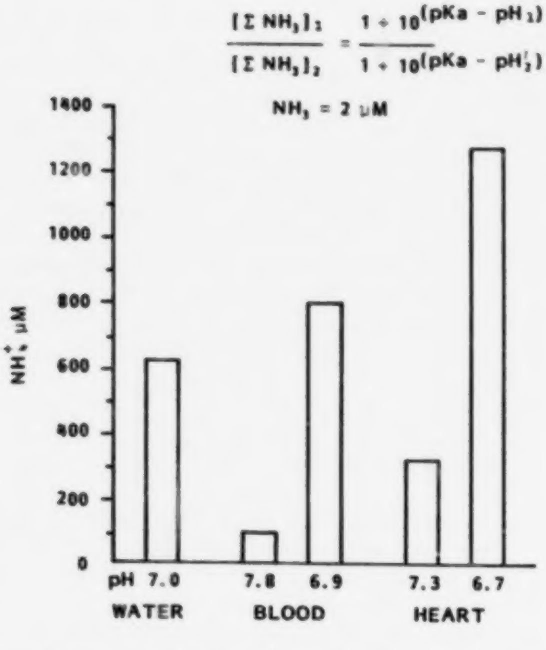


Figure 2. pH dependence of $NH_3:NH_4^+$ ratio in water, blood, and heart. pH values taken from Heisler (1980) for normal and acidotic conditions in fish.

AMMONIA EXCRETION

The excretion of ammonia by fish is variable, depending on the state of the animal, the environmental conditions, and the species. Ammonia excretion tripled in sockeye salmon following daily feeding (Brett and Zala, 1975) but remained low and unchanging during 22 days of starvation. In freshwater fish ammonia excretion increases in response to exercise (Sukumaren and Kutty, 1977; Holeton et al. 1983), long-term acid exposure (McDonald and Wood, 1981; Ultsch et al., 1981), hypercapnia (Claiborne and Heisler, 1984) and NH_4Cl infusion (Hillaby and Randall, 1979). In contrast, short-term exposure to acid or alkaline water caused a decrease in ammonia excretion in trout (Wright and Wood, 1984). It is not known if these changes in excretion reflect changes in the rate of ammonia production or in the ammonia content of the body. The ammonia content of fish is likely to be the equivalent of the ammonia excreted in about 2 hours, most of the ammonia being in the tissues with a lower pH, like muscle. Blood levels are around 0.200 to 0.300 mMol, but muscle at a lower pH may have concentrations up to 1 mMol. Thus a kg fish may contain about 0.500 to 0.700 mMol of ammonia and have an excretion rate of about 0.300 mMol per hour. There is increased ammonia production in muscle during exercise (Driedzic and Hochachka, 1976). Ammonia excretion by the dogfish in seawater is unaffected by temperature change, exercise, hyperoxia, hypercapnia, or the infusion of either HCl or $NaHCO_3$ or anything that induces acid-base stress (see Heisler, 1984, for review). This is surprising, because many of these changes affect pH and therefore would be expected to alter the ammonia content of body compartments and therefore ammonia excretion.

There is an elevation in blood ammonia during starvation (Hillaby and Randall, 1979; Morii, 1979), which is perhaps surprising because ammonia excretion declines (Brett and Zala, 1975). Blood ammonia levels also rise with increases in temperature (Fauconneau and Luquet, 1979) and with higher ammonia concentrations in the water (Fromm and Gillette, 1968). Exposure of fish either to air (Gordon, 1970) or to increased ammonia levels in water (Fromm, 1970; Guerin-Ancey, 1976), raises blood ammonia levels and reduces ammonia excretion and this is associated with a rise in urea production in many, but not all fish. Unlike the above studies, Buckley et al. (1979) found no change in blood total ammonia when coho salmon were exposed to elevated ammonia levels in the environment. They did observe a significant rise in plasma sodium, however, indicating some coupling between sodium uptake and ammonia excretion (see below).

AMMONIA EXCRETION ACROSS GILLS

Most of the ammonia produced by the fish is excreted across the gills. Oxygen, carbon dioxide, ions and water also are transferred across the gills. The movement of ammonia gas is largely independent of the transfer of other molecules, and is a function of the unionized ammonia gradient. Ammonia entry into the fish depends on this gradient (Wuhrmann et al., 1947; Wuhrmann and Woker, 1948; Fromm and Gillette, 1968). The rate of loss also depends on the NH_3 gradient, in most instances (Hillaby and Randall, 1979; Kormanik and Cameron 1981; Cameron and Heisler, 1983). The excretion of ammonium ion is strongly coupled to the movement of other ions. Membranes, including the gills, are not very permeable to cations like ammonium ion. Ammonium ion can displace potassium in many membrane processes, for example in squid giant axon (Binstock and Lecar, 1969), and this is the probable reason that elevated ammonia causes convulsions in so many vertebrates. In fish gills it is possible that NH_4^+ can substitute for potassium in oubain-sensitive sodium/potassium exchange and also substitute for protons in amiloride sensitive Na^+/H^+ exchange, the former moving ammonium from blood into the gill epithelium, the latter exchanging ammonium for sodium on the outer surface of the gill epithelium (Maetz and Garcia-Romeu, 1964; Evans, 1977; Girard and Payan, 1980; Wright and Wood, 1984). Either acid conditions or amiloride in the water inhibit Na^+ influx across the gills and both these conditions result in a reduction of ammonia excretion (Wright and Wood, 1984) and ammonia infusion will stimulate sodium influx even in seawater fish (Evans, 1977). It is interesting to note that ammonia excretion was maintained, even in the face of a calculated reversed ammonia gas gradient, presumably by sodium/ammonium exchange. Cameron and Heisler (1983) could account for ammonia excretion in trout, under most conditions, by the diffusion of unionized ammonia, but in the presence of high external ammonia, sodium/ammonium exchange may counter balance the diffusive uptake of ammonia gas. Indeed, this would explain the unchanging blood ammonia but increased sodium levels in coho salmon exposed to elevated water ammonia (Buckley et al. 1979). Ammonium ion efflux and sodium influx must be quantified to determine the exact relationship between sodium and ammonium movements. It is relatively easy to determine sodium influx using isotopes but it is difficult to determine NH_4^+ efflux. Methods exist for measuring total ammonia excretion from measurements of total ammonia in blood or water. If the pH is known in both blood and water, then the ammonia and ammonium ion concentrations on both sides of the gills can be calculated and the ammonia gas and ammonium ion differences across the gills estimated. Wright and Wood (1984) did this for rainbow trout exposed to a variety of acid and alkaline conditions or following inhibition of sodium uptake with amiloride. They observed a positive correlation between total ammonia excretion and the ammonia gas gradient. If the ammonia gas excretion is subtracted from total ammonia excretion, then there was an approximate relationship between sodium influx and ammonium ion efflux.

The difficulty with this approach is that the exact pH of water and blood on either side of the gill epithelium is not known. The pH of afferent and efferent blood can be measured and the mean of these two may be an approximation of the pH of blood in the gills. Water pH is equally difficult to determine. Firstly there are undoubtedly boundary layers next to the gill surface and this will contain concentration gradients; secondly there will be longitudinal gradients as material is added to the gill water.

Substances added to the water that will affect pH are CO_2, ammonia and ammonium ion, and protons. There is no evidence that CO_2 hydration occurs at a catalysed rate in gill water; in as much as the water is only in contact with the gill for about 100 to 400 msec. (Randall, 1982), and at low pH the uncatalysed CO_2 reaction takes several seconds, any effect of CO_2 on water pH will occur after the water has passed over the gills. Any excretion of protons will have an immediate effect lowering pH, whereas excretion of ammonia gas will raise pH. Conversely, excretion of ammonium ion will lower pH and, because ammonia/ammonium reactions are rapid, they will influence water pH at the gills and therefore ammonia excretion. One might predict that, under most conditions, ammonia excretion will exceed both ammonium ion and proton excretion, thus the effect will be to raise the pH of the water in contact with the gills. Although the amount of CO_2 excreted will far exceed that of ammonia, there will not be an appreciable reduction in water pH until the water has left the gills. It is clear a more detailed understanding of ammonia excretion requires a more detailed analysis of pH gradients across the gills.

Wright and Wood (1984) measured ammonia excretion in trout exposed to a variety of water pH conditions, and they found that sodium influx was zero at pH 4.1. If it is assumed that under these conditions ammonium/sodium exchange is also zero, then all ammonia excreted must have been due to unionized ammonia diffusion. The unionized ammonia gradient was calculated from measurements of pH and total ammonia content in water and blood. The calculated permeation coefficient for ammonia diffusion across the trout gill was 65% of that calculated by Cameron and Heisler (1983). The unionized ammonia excretion was then calculated for trout under other conditions using this permeation coefficient and the estimated NH_3 gradients. The ammonium ion excretion was then determined by subtracting the NH_3 excretion from the measured total ammonia excretion.

A plot of ammonium ion excretion against sodium influx for trout under a variety of conditions is shown in Figure 3a. Considering only control and acid exposed fish there is a close 1 to 1 correlation between sodium influx and ammonium excretion (Figure 3b), indicating a tight coupling of sodium and ammonium exchange. Under alkaline conditions, however, the coupling was not obvious although clearly sodium influx was reduced. If it is assumed that under all conditions there is a 1 to 1 relationship between sodium and ammonium ion flux (Figure 3c) then under alkaline conditions there must have been an underestimate of the unionized ammonia excretion (Figure 3a).

The estimation of unionized ammonia excretion depended on the assumption that pH in the bulk medium was the same as that at the surface of the gills. If, under alkaline conditions, there is still a tight coupling of ammonium ion and sodium exchange--that is, ammonium efflux equals sodium influx (see Figure 3c), we can estimate unionized ammonia excretion by subtracting ammonium ion excretion from total ammonia excretion. Table 1 lists the calculated NH_3 and NH_4^+ movements across the gills, note that in some cases there is an excretion of ammonium ion but an uptake of ammonia by the fish. We have determined the effect of ammonia transfer on water pH at the gills from these fluxes, the buffering capacity of the water, and the equilibrium constants for the NH_3/NH_4^+ reaction (Thurston et al., 1979).

The effects of ammonia movements on water pH (Table 1) are small, especially at water pH between 6.6 and 8.1. At water pH of 8.7 and 9.5, however, ammonia transfer has some effect on the pH of water as it passes over the gills. The NH_3 gradient, and therefore NH_3 excretion in the study of Wright and Wood (1984), was based on the pH of the bulk phase water rather than the pH of water at the gill surface. The fact that the pH of water at the gill surface was affected by ammonia transfer at these high water pH levels, may have led to an error in NH_3 excretion and

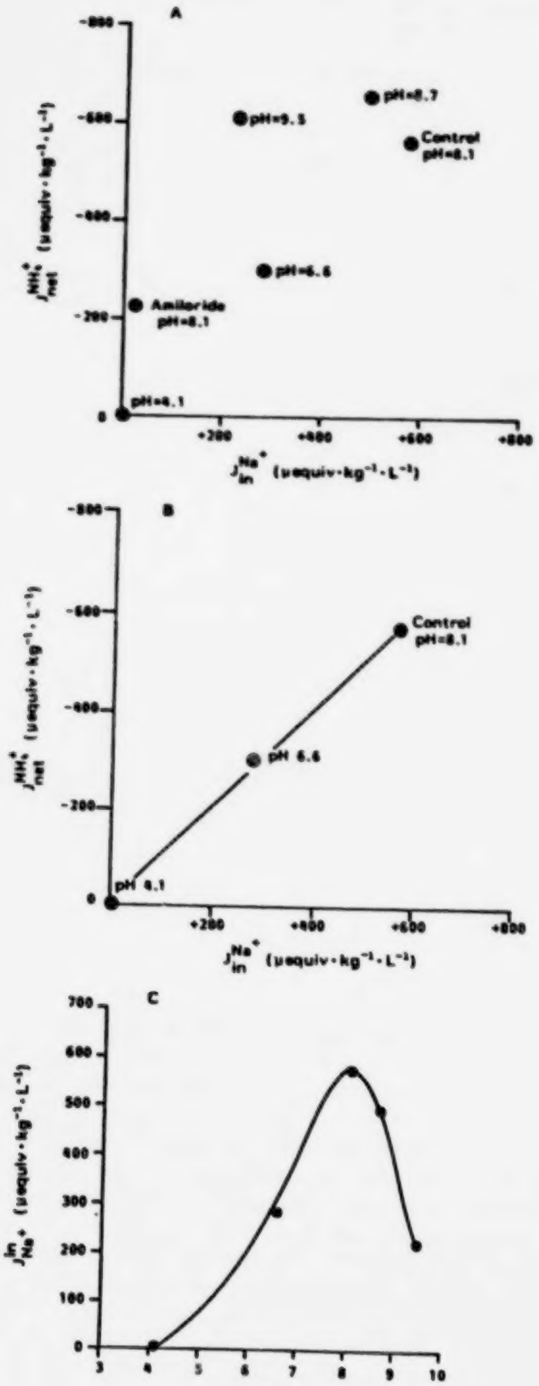


Figure 3. Relationship of Na^+ influx to NH_4^+ efflux, and Na^+ influx to pH. Each dot represents mean of at least 8 fish.

TABLE 1. ANALYSIS OF AMMONIA EXCRETION IN TERMS OF Na^+/HN_4^+ EXCHANGE AND WATER pH NEXT TO THE GILL.

Water	M_{amm}[1]	$J^{out}_{NH_3}$[1]	$J^{out}_{NH_4^+}$[2]	ΔpH[3]
Acid pH 4.1	280.99	280.99	0	.06
Moderate acid pH 6.6	403.09	118.20	284.89	.02
Control pH 8.1	393.58	-179.73	573.31	-.03
Amiloride pH 8.1	232.00	193.04	38.96	+.03
Moderate alkaline pH 8.7	261.35	-231.00	492.35	-.10
Severe alkaline pH 9.5	67.19	-155.22	222.76	-.10

1. measured in $\mu mol.kg^{-1}.h^{-1}$
2. measured in $\mu equiv.kg^{-1}.h^{-1}$
3. ΔpH = pH calculated -pH measured

therefore an overestimate of ammonium ion excretion. As a result, Wright and Wood (1984) did not observe a one to one relationship between ammonium ion and sodium flux at these elevated water pH levels (Figure 3a).

Carbon dioxide in the water affects ammonia toxicity; if CO_2 levels are raised, total ammonia toxicity is decreased (Alabaster and Herbert, 1954). CO_2 causes a fall in pH and decreases the proportion of unionized ammonia in solution. The unionized form has a greater toxic effect because ammonia must enter the fish to exert its toxic action and lipid membranes are much more permeable to unionized ammonia (Wuhrmann et al., 1947; Wuhrmann and Woker, 1948; Thurston et al., 1981). Thus the reduction in unionized ammonia associated with the fall in water pH caused by the rise in CO_2 decreases total ammonia toxicity. Lloyd and Herbert (1960) found, however, that although total ammonia toxicity was reduced at high CO_2 levels, the inverse was true when considering unionized ammonia alone. More unionized ammonia is required in low CO_2-high pH water to exert the same toxic effect as seen in fish in high CO_2-low pH water. The explanation presented by Lloyd and Herbert (1960) for the decreased toxicity of unionized ammonia in low CO_2 water was that CO_2 excretion across the gills would reduce pH and therefore the concentration of unionized ammonia in water flowing over the gills. This is consistent with our conclusions of the effects of CO_2 at high pH, but not at water pH levels of below 8.10. Another possible explanation is that the blood pH of the fish also varied inversely with the CO_2 content of the water such that the total ammonia content of the blood decreases with the CO_2 in water pH in Lloyd and Herbert's experiments, and both these factors are known to reduce blood pH in fish (Janssen and Randall, 1975; Randall et al., 1976; Heisler, 1980). The fish in Lloyd and Herbert's experiments were exposed to water of different pH and CO_2 levels for 18 hours and the blood pH of these fish was probably inversely related to CO_2 levels in the water at the time of ammonia exposure. Blood pH will be an important determinant of blood total ammonia levels and this in turn is an important factor in its toxic action (Hillaby and Randall, 1979). Blood pH is probably decreased with increasing CO_2 levels in the water, and this causes an increase in the blood total ammonia levels for a given unionized ammonia concentration. This could account for the differences in unionized ammonia toxicity observed. What is required is accurate measurement of pH in both blood and water and therefore NH_3 gradients across the gill epithelium.

REFERENCES

Alabaster, J.S., and D.W. M. Herbert. 1954. Influence of carbon dioxide on the toxicity of ammonia. Nature 174:404.

Binstock, L., and H. Lecar. 1969. Ammonium ion currents in the squid giant axon. J. Gen. Physiol. 53. p. 342-361.

Boutilier, R.G., T.A. Heming, and G.K. Iwama. 1984. Appendix: Physiochemical parameters for use in Fish Respiratory Physiology. Appendix: Fish Physiology, Vol XA ed. W.S. Hoar and D.J. Randall. Academic Press Inc.

Brett, J.R., and C.A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (Oncorhynchus nerka) under controlled conditions. J. Fish. Res. Biol. Can. 32:2479-2486.

Buckley, J.A., C.M. Whitmore., and B.D. Liming. 1979. Effects of prolonged exposure to ammonia on the blood and liver glycogen of coho salmon (Oncorhynchus kisutch). Comp. Biochem. Physiol. 63C:297-303.

Cameron, J.N., and N. Heisler. 1983. Studies of ammonia in the rainbow trout: physio-chemical parameters, acid-base behavior, and respiratory clearance. J. Exp. Biol. 105:107-125.

Claiborne, J.B., and N. Heisler. 1984. Acid-base regulation in the carp (Cyprinus carpio) during and after exposure to environmental hypercapnia. J. Exp. Biol. 108:25-43.

Driedzic, W.R., and P.W. Hochachka. 1976. Control of energy metabolism in fish white muscle. Am. J. Physiol. 230:579-582.

Evans, D.H. 1977. Further evidence for Na/NH_4 exchange in marine teleost fish. J. exp. Biol. 70:213-220.

Fauconneau, B., and P. Luquet. 1979. Influence d'une elevation de temperature sur l'evolution de l'aminoacidemie et de l'ammoniemie apres le repas chez la truite arc en ciel (Salmo gairdneri R.). Ann. Biol. anim. Bioch. Biophys. 19(4A):1063-1079.

Florkin, M., and G. Duchateau. 1943. Les formes du systeme enzymatique de l'uricalyse et l'evolution du catabolisme purique chez les animaux. Arch. Int. Physiol. 53:267-307.

Forster, R.P., and L. Goldstein. 1969. Formation of excretory products. In: Fish Physiology. Eds. W.S. Hoar and D.J. Randall, Academic Press, NY. Vol I., pp. 313-350.

Fromm, P.O., and J.R. Gillette. 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. 26:887-896.

Fromm, P.O. 1970. Section III. Effect of ammonia on trout and goldfish. In: Toxic action of water soluble pollutants on freshwater fish. EPA Water Pollution Control Research Series. 18050 DST 12/70 pp. 9-22.

Girard, J.P., and P. Payan. 1980. Ion exchanges through respiratory and chloride cells in freshwater and seawater adapted teleosteans. Am. J. Physiol. 238: (Regulatory Integrative Comp. Physiol. 7) R260-R268.

Gordon, M.S. 1970. Patterns of nitrogen excretion in amphibious fishes. Urea Kidney, Proc. Int. Colloq. 1968:238-242.

Gregory, R.B. 1977. Synthesis and total excretion of waste nitrogen by fish of the Periophthalmus (mudskipper) and Scartelass families. Comp. Biochem. Physiol. 57A:33-36.

Guerin-Ancey, O. 1976. Etude experimentale de l'excretion azotee du bar (Dicentrarchus labrax) en cours de croissance. II. Effets du jeune sur l'excretion d'ammonia det d'uree. (Experimental study of the nitrogen excretion of bass (Dicentrarchus labrax) during growth. II. Effects of starvation on the excretion of ammonia and urea). Aquaculture 9(2):187-194.

Heisler, N. 1980. Regulation of the acid-base status in fishes. In: Environmental physiology of fishes. Ed. M.A. Ali, Plenum Publishing Co., NY.

Heisler, N. 1984. Acid-base regulation in fishes. In: Fish Physiology Vol. XA, ed. W.S. Hoar and D.J. Randall. Academic Press Inc., NY.

Hillaby, B.A., and D.J. Randall. 1979. Acute ammonia toxicity and ammonia excretion in rainbow trout (Salmo gairdneri). J. Fish. Res. Biol. Canada 36:(6)621-629.

Holeton, G.F., P. Neumann, and N. Heisler. 1983. Branchial ion exchange and acid-base regulation after strenuous exercise in rainbow trout. Resp. Physiol. 51,303-318.

Janssen, R.G., and D.J. Randall. 1975. The effects of changes in pH and P_{CO_2} in blood and water on breathing in rainbow trout, *Salmo gairdneri*. Resp. Physiol. 25:235-245.

Kormanik, G.A., and J.N. Cameron. 1981. Ammonia excretion in the FW catfish: the role of diffusion. Amer. Soc. Zool. 21(4):1042.

Levi, G., G. Morisi, A. Coletti, and R. Catanzaro. 1974. Free amino acids in fish brain: normal levels and changes upon exposure to high ammonia concentrations *in vivo*, and upon incubation of brain slices. Comp. Biochem. Physiol. 49A:623-636.

Lloyd, R., and D.W.M. Herbert. 1960. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). Ann. appl. Biol. 48:399-404.

Maetz J., and F. Garcia-Romeu. 1964. The mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus* II Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. J. Gen. Physiol. 47:1209-1227.

McDonald, D.G., and C.M. Wood. 1981. Branchial and renal acid and ion fluxes in the rainbow trout at low environmental pH. J. Exp. Biol. 93:101-118.

Morii, H. 1979. Changes with time of ammonia and urea concentrations in the blood and tissue of mudskipper fish, *Periophthalmus cantonensis* and *Boleophthalmus pectinirostris*, kept in water and on land. Comp. Biochem. Physiol. 64A:235-243.

Randall, D.J. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol. 100:275-288.

Randall, D.J., N. Heisler and F. Drees. 1976. Ventilatory response to hypercapnia in the larger spotted dogfish *Scyliorhinus stellaris*. Am. J. Physiol. 230:590-594.

Sukumaran, N., and M.N. Kutty. 1977. Oxygen consumption and ammonia excretion in the catfish *Mystus armatus*, with special reference to swimming speed and ambient oxygen. Proc. Indian. Acad. Sci. 86B:195-206.

Thurston, R.V., R.C. Russo, and K. Emerson. 1979. Aqueous ammonia equilibrium-tabulation of percent un-ionized ammonia. U.S. Environmental Protection Agency, Duluth, Minnesota. EPA-600/3-79-091.

Thurston, R.V., R.C. Russo, and G.A. Vinogradov. 1981. Ammonia toxicity to fishes: effect of pH on the toxicity of the un-ionized ammonia species. Environ. Sci. Technol. 15(7): 837-840.

Trussell, R.P. 1972. The percent unionized ammonia in aqueous ammonia solutions at diffeent pH levels and temperatures. J. Fish. Res. Board Can. 29(10):1505-1510.

Ultsch, G.R., D.C. Jackson, and R. Moalli. 1981. Metabolic oxygen conformity among lower vertebrates: the toadfish revisited. J. Comp. Physiol. 142:439-443.

Walton, M.J., and C.B. Cowey. 1977. Aspects of ammoniogenesis in rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 57B:143-149.

Watts, R.L., and D.C. Watts. 1974. Nitrogen metabolism in fishes. In: Chemical zoology. Eds. M. Florkin and B.T. Scheer, Vol. VIII, Academic Press, NY. pp. 369-446.

Webb, J.T., and G.W. Brown, Jr. 1976. Some properties and occurrence of glutamine synthetase in fish. Comp. Biochem. Physiol. 54B:171-175.

Wright, P.A., and C.H. Wood. 1984. An analysis of branchial ammonia excretion in the freshwater rainbow trout: effects of environmental pH change and sodium uptake blockade. J. Exp. Biol. (in press).

Wuhrmann, K., and Woker, H. 1948. Contributions to the toxicology of fishes. II Experimental investigations on ammonia - and hydrocyanic acid poisoning. Translation. Schweiz. Z. Hydrol. 11:210-244.

Wuhrmann, K., F. Zehender, and H. Woker. 1947. Biological significance for fisheries of ammonium-ion and ammonia content of flowing bodies of water. *Translation of*: "Uber die fischereibiologische Bedeutung des Ammonium-und Ammoniakgehaltes fliessender Gewässer". Vierteljahrsschrift der Naturf. Gesellschaft in Zurich 92:198-204.

THE USE OF FISH BEHAVIOR IN COMPARING TOXIC EFFECTS OF THREE CHEMICALS

by

M.G. Henry[1]

ABSTRACT

The behavioral effects of three chemicals are compared using a method developed to monitor changes in social groups of bluegill, Lepomis macrochirus. Ten behaviors were observed once daily for 96-hr before and after treatment. Methyl parathion induced hyperactivity followed by changes in frequency of comfort movements. Copper sulfate disrupted respiration, but the most striking impact was an increase in aggression caused by chlordane. The distribution of toxicant-related responses was related to social rank in each of the treated hierarchies. The dominant and subordinate fish were most affected. The best indicator of toxicant effect across rank was respiratory in nature, because coughs increased in all fish exposed to each of the three compounds. This assay was sensitive enough to detect behavioral alterations at concentrations well below lethal levels. Behavioral bioassays, if selected to relate to important life history characteristics of the species of interest, could provide useful ecological links for interpreting laboratory effects and verifying them in the field.

INTRODUCTION

Assessing the toxicity associated with environmental contaminants is chiefly a biological problem because their impact on aquatic organisms is at the heart of the hazard evaluation process. Despite good intentions and sound hypotheses, however, the reality persists that our ability to relate laboratory effects to field effects is very limited. Classical toxicity tests examining mortality, changes in growth, and effects on reproduction have used single species and single compounds under sterile test conditions. The purpose they serve is that these tests produce toxicity values that are comparable in nature due to the use of standardized test methodology. Despite this

---

[1] Columbia National Fisheries Research Laboratory, Rt. 1, Columbia, MO 65201
Present address: Great Lakes Fishery Laboratory, 1451 Green Road, Ann Arbor, MI 48105

advantage, the issue of ecological significance remains. Ecological aspects of an organism's life history, such as its behavior, need to be incorporated into the development and standardization of testing protocols.

Behavior is considered the organismal level manifestation of the physiologically and environmentally influenced state of the animal. Behavior mediates survival and reproduction by influencing predator-prey interactions, feeding efficiency, swimming performance, courtship, nest digging, etc. If key behaviors are altered by a contaminant, then ultimately survival and reproduction could decline. Measurement of behavioral parameters can assist us in interpreting toxicant effects within an ecological framework. In addition, data from behavioral bioassays may aid us in designing more focused field studies so that verification of laboratory effects is possible.

Keeping in mind the issues of ecological significance, cost effectiveness, standardized methodology, and applicability over a range of compound classes, we developed this test. Bluegill (Lepomis macrochirus) were used because they are ubiquitous throughout North America, have been used in classical toxicity tests, and are behaviorally diverse. The objectives of this research were to compare the behavioral effects of three chemically distinct compounds and evaluate the overall utility of this test method.

MATERIALS AND METHODS

Ten different behaviors were monitored in each established population consisting of five adult bluegill. Each behavior related either to body maintenance or social interaction. Daily observations (0.5 hr/tank) were made directly and the frequencies of each behavior were recorded by hand. Individual fish were recognized on the basis of size, color and natural markings. No tags, brands or fin clips were utilized in the event that they would interfere with normal behavior patterns.

Fish were randomly assigned to four 295L aquaria, equipped with plants (Elodea) and gravel to simulate some of the characteristics of a natural habitat. Tanks were monitored for 96-hr before and after addition of the toxicant. Each flow-through system utilized a modified Mount and Brungs (1967) proportional diluter to deliver a different toxicant concentration to each test tank. The lowest concentration selected for each toxicant examined was based on a level (if available in the literature) that approximated the low end of the Maximum Allowable Toxicant Concentration (MATC). The middle concentration approximated the upper end of the MATC and the high concentration approached the reported Lethal Concentration (LC50). If a MATC was not available, a geometric progression from the LC50 was used instead. A well water control was incorporated to monitor changes through time. The purpose of selecting levels based on MATCs and LC50s was to facilitate comparison of the sensitivity of this new behavioral technique to endpoints obtained through more classically accepted protocols.

Three separate experiments were conducted, each including two complete replications. The three experiments were based on the behavioral evaluation of three chemically distinct compounds: methyl parathion - an organophosphate insecticide, copper sulfate - a heavy metal, and chlordane - an organochlorine insecticide. Water concentrations were determined using gas-liquid chromatography or atomic absorption spectrophotometry.

Because the number of chemicals in the United States requiring screening is so vast, standardization of new methods is crucial so that industry, universities and government agencies can utilize them. In over 40 replications of this behavioral bioassay, methods standardization was possible so that the whole procedure could be completed in 17 to 21 days. This time period is considerably less than that required in partial-chronic tests, thereby increasing cost efficiency without sacrificing sensitivity. A more detailed methods description and results/discussion can be found in papers by Henry and Atchison (1979a, 1979b, 1984, in review).

For comparative purposes, four behaviors affected by the middle concentration of each chemical have been selected for discussion. Two behaviors relate to respiratory processes and two relate to social interaction, specifically aggressive behaviors used in hierarchy establishment and maintenance.

The two respiratory behaviors that were examined were coughs and yawns. Coughs (Henry and Atchison 1979a, 1979b, 1984) were identified as the rapid, repeated opening and closing of the mouth and opercular coverings, accompanied by partial extension of the paired fins. A yawn was conversely recognized as a singular event typified by maximal opening of the mouth and opercular covering accompanied by hyperextension of all fins, both paired and medial.

The two socially induced behaviors we will discuss are nips (bites) and nudges (contact between fish, the aggressor touching the recipient with a closed mouth). Both of these behaviors are aggressive in nature.

Data were transformed ($\sqrt{x+1}$) and analyzed using analysis of variance and least significant difference determinations (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The postures and forms of behaviors monitored did not change in the presence of the toxicants used; however, frequency- and hierarchy-related distribution throughout the test population was altered.

In general, hyperactivity was noticeable in all fish exposed to methyl parathion. Two comfort movements (s-jerks and fin flicks) were most altered in frequency, increasing significantly ($P=0.001$) over control levels once methyl parathion was introduced (Henry and Atchison 1984). Coughs also increased significantly ($P=0.01$) in the presence of the toxicant (Table 1). Copper sulfate did not induce hyperactivity but dramatically influenced respiratory disruptions, increasing the frequency of coughs ($P=0.01$) and yawns ($P=0.01$) well beyond levels observed during the pre-exposure period. Chlordane produced large elevations ($P=0.01$) in aggressive behaviors. The more intense manifestations, nips, became so frequent that the most subordinate fish was killed by the dominant in the 0.044 mg/L treatment.

TABLE 1. MEAN FREQUENCIES OF FOUR BEHAVIORS EXAMINED BEFORE AND AFTER ADDITION OF THREE CONTAMINANTS

Toxicant (middle concentration)	Behavior			
	Cough	Yawn	Nip	Nudge
	C:T[a]	C:T	C:T	C:T
Methyl parathion (0.03 mg/L)	19.0/29.5	7.0/8.5	39.0/125	8.0/1.5
Copper sulfate (0.057 mg/L)	9.0/72.0	6.0/31.0	31.0/71.5	4.0/9.0
Chlordane (0.002 mg/L)	13.5/48.0	7.0/41.0	31.0/78.5	6.5/1.0

[a] C:T represents mean control values compared to treatment values.

Differential response associated with social rank occurred at every concentration for each toxicant examined. The dominant and the most subordinate fish were usually affected to the greatest extent. Because fish of those two ranks are initially under the greatest amount of social stress (Sparks et al. 1972, Noakes and Leatherland 1977), the combination of social stress and toxicant stress produced a more extreme response than was seen in fish of intermediate rank. The dominant and subordinate fish of each experiment are compared in Table 2 before and after toxicant addition. Subordinate fish suffered more respiratory disruptions than dominants; however, aggressive behaviors were significantly altered in top ranking individuals. Fish were affected differently, therefore, depending on hierarchy position but all responded to the presence of the toxicants. Coughs, although most altered in subordinates, universally increased in fish of every rank exposed to each of the three contaminants. If one behavior had to be selected for cursory examination across ranks, cough frequency would be the best overall indication of effect.

We know that the toxic mode of action for methyl parathion is inhibition of cholinesterase at synaptic and neuromuscular junctions. Severe inhibition of this enzyme often results in induction of hyperactivity and muscular spasms (Brown 1978). As was previously mentioned, hyperactivity was observed in fish exposed to methyl parathion. Copper, on the other hand, has been associated with causing changes in gill structure (Anderson and Spear 1980). Increases in coughs and yawns relate to this suspected underlying change in physiology and morphology. Chlordane also disrupts respiration. However, it seems to do so by creating anaerobic conditions in gill tissue via inhibition of acetylcholinesterase (Verma et al. 1981). Thus it is both a neurotoxin and respiratory disrupter. Coughs and yawns increased in fish exposed to chlordane but aggression did as well.

These underlying physiological mechanisms associated with the induction of toxic responses can be observed at an organismal level in the altered frequency of behaviors described above. Thus, this technique appears to be not only sensitive but applicable across different classes of chemicals. It requires minimal equipment, can be conducted in 17 to 21 days, and is ecologically related to maintenance of social groups of bluegill.

This test is, however, only appropriate for examining species that form hierarchies. It is not appropriate for solitary predators, schooling fishes, etc. Consequently, use and standardization of other behavioral tests such as predator-prey, swimming performance or avoidance tests could be utilized depending on the life history characteristics of the species of interest. For example, swimming performance should be evaluated when anadromous fish are exposed to a toxicant. Sufficient swimming stamina allows them to reach nursery spawning areas and if altered could reduce reproduction.

Behavioral bioassays, used in conjunction with other approaches such as acute, chronic, and partial chronic tests, residue dynamic studies, and physiological or biochemical assays will help us more realistically evaluate chemicals and set ecologically meaningful water quality standards.

REFERENCES

Anderson, P. D., and P. A. Spear. 1980. Copper pharmaco-kinetics in fish gills-II. Body size relationships for accumulation and tolerance. Water Research 14(8):1107-1111.

TABLE 2. MEAN FREQUENCIES OF FOUR BEHAVIORS PERFORMED BY MOST DOMINANT AND SUBORDINATE RANKED FISH EXPOSED TO THREE TOXICANTS

	Behavior			
Toxicant	Cough	Yawn	Nip	Nudge
Methyl parathion				
(0.03 mg/L)	D:S	D:S	D:S	D:S
Control	0.5/8.0	0.0/5.0	14.5/0.0	4.0/0.0
Treated	4.5/16.0	1.0/3.5	4.5/0.0	0.5/0.0
Copper sulfate				
(0.057 mg/L)				
Control	3.5/3.0	0.0/2.5	16.5/0.5	0.5/1.0
Treated	14.5/30.5	7.0/12.0	52.5/1.5	1.0/0.0
Chlordane				
(0.002 mg/L)				
Control	2.0/4.5	1.0/2.0	19.0/0.0	0.25/0.5
Treated	14.0/22.0	10.5/19.5	62.5/0.0	0.25/0.25

Brown, A. W. A. 1978. Ecology of pesticides. John Wiley and Sons, New York, New York.

Henry, M. G., and G. J. Atchison. 1979a. Behavioral changes in bluegill (Lepomis macrochirus) as indicators of sublethal effects of metals. Environmental Biology of Fishes 4:37-42.

Henry, M. G., and G. J. Atchison. 1979b. Influence of social rank on the behavior of bluegill Lepomis macrochirus Rafinesque, exposed to sublethal concentrations of cadmium and zinc. Journal of Fish Biology 15:309-315.

Henry, M. G., and G. J. Atchison. 1984. Behavioral effects of methyl parathion on social groups of bluegill (Lepomis macrochirus). Environmental Toxicology and Chemistry 3:399-408.

Henry, M. G., and G. J. Atchison. In review. The effects of copper on the behavior of bluegill, Lepomis macrochirus. Transactions of the American Fisheries Society.

Noakes, D. L. G., and J. F. Leatherland. 1977. Social dominance and interrenal cell activity in rainbow trout, Salmo gairdneri. Environmental Biology of Fishes 2:131-136.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research 1:21-29.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames, Iowa.

Sparks, R. E., W. T. Waller, and J. Cairns, Jr. 1972. Effects of shelters on the resistance of dominant and submissive bluegills (Lepomis macrochirus) to a lethal concentration of zinc. Journal of the Fisheries Research Board of Canada 29:1356-1358.

Verma, S. R., I. P. Tonk, A. K. Gupta, and R. C. Dalela. 1981. In-vivo enzymatic alterations in certain tissues of Saccobranchus fossilis following exposure to four toxic substances. Environmental Pollution Series A: Ecological Biology 26(2):121-128.

RESISTANCE OF AQUATIC ANIMALS TO ORGANOPHOSPHORUS PESTICIDES AND ITS MECHANISMS

by

V.I. Kozlovskaya, G.M. Chuyko, L.N. Lapkina
and V.A. Nepomnyashchikh[1]

ABSTRACT

Aquatic animals possess varying resistance to organophosphorus pesticides. Fish are more resistant than invertebrates. The toxicity of organophosphorus pesticides is caused by their ability to inhibit cholinesterase, primarily acetylcholinesterase of the nervous system. This leads to a disruption of the nervous system. The molecular forms of acetylcholinesterase from various animal species differ in terms of sensitivity to organophosphorus compounds. A dependence between the sensitivity of an enzyme to organophosphorus pesticides and the toxicity of preparations for an organism is apparent between invertebrates and fish, among species close in taxonomic position as well as in individuals in the same species for various toxic substances. The resistance of aquatic animals, like ground insects and mammals, depends upon the sensitivity of their cholinesterase. But this is not the only mechanism of resistance. In the resistance of animals to organophosphorus pesticides, a definite role is also played by the processes of metabolism and permeability.

INTRODUCTION AND DISCUSSION

Water pollution by pesticides, including organophosphorus compounds, has an adverse effect on hydrobionts. To effectively monitor these agents in water and forecast their effect on fauna, it is necessary to determine the resistance of different aquatic animals to pesticides and investigate its mechanisms.

Aquatic animals differ in resistance to this group of compounds. Thus, among the 12 species of leeches referable to 2 orders and 4 families, there are species with high (Protoclepsis tessulata, Hemiclepsis marginata) and

---

[1] Institute of Biology of Inland Waters, USSR Academy of Sciences, Borok, USSR

low (*Caspiobdella fadejewi*, *Herpobdella nigricollis*) resistance (Lopkina and Flerov 1979). Among crustaceans and mollusks, the water flea (*Daphnia pulex*), hog slater (*Asellus aquaticus*) and large snail (*Limnaea stagnalis*) are the least resistant. The trumpet snail (*Planorbis corneus*) dies at high concentrations, the same as fish including crucian carp (*Carassius carassius*), roach (*Rutilus rutilus*) and zope (*Abramic ballerus*). The carp (*Cyprinus carpio*) is the most resistant species. Chlorofos toxicity is 100,000 times higher for the water flea than the carp. There are also fish species with low resistance. They are representatives of the Salmonidae (*S. irrideus*) and Percidae (*P. fluviatilis*) families (Table 1 and 2). Our data on toxicity of organophosphorus pesticides for aquatic animals are essentially in agreement with the data in surveys [Hogan and Knowles 1968, Chuyko et al. 1983] prepared on the basis of results of American studies.

Organophosphorus compounds have a dissimilar effect on different stages of the life cycle of aquatic animals. In leeches, newly hatched young specimens that have not begun independent feeding are the least resistant. (48-h LC_5 are 0.003 and 0.03 mg/ℓ for *Caspiobdella fadejevi* and *Piscicola geometra*, respectively. Leech cocoons are the most resistant to toxicants. Chlorofos in concentrations of 0.5 and 1 mg/ℓ, with 48-h exposure, which has a devastating effect on adult specimens, is virtually harmless for cocoons. Resistance of eggs in cocoons is at a maximum at the early stages of embryonic development and it diminishes by the end of development (Table 3) (Lapkina 1983).

For fish, organophosphorus pesticides are the most hazardous during the embryonic (from the time of fertilization to cleavage and at organogenesis stage), larval, and young fry periods of development. In concentrations of 0.01-0.001 mg/ℓ, metaphos, phosalone, methylnitrophos, iosophos, and sayfos impair embryogenesis and cause hatching of malformed larvae. At the prelarval stage, exposure to a weaker toxic agent does not reveal visible disturbances; however, by the larval stage there are pathological deviations of development. One of the distinctions of effect of organophosphorus compounds on embryogenesis is slower embryonic development, which is associated with loss of a large number of embryos.

TABLE 1. TOXICITY OF ORGANOPHOSPHORUS PESTICIDES FOR AQUATIC INVERTEBRATES, 48-h EXPOSURE AND TEMPERATURE OF 18-21°C, mg/ℓ

Pesticides and Organisms	Stage, size, mm	Maximum tolerated concentration (MTC)	LC_{50}	LC_{100}
Chlorofos				
Leeches				
Protoclepsis tessulata	15	5	105	300
Hemiclepsis marginata	15-22	20	100	300

TABLE 1. (Continued)

Pesticides and Organisms	Stage, size, mm	Maximum tolerated concentration (MTC)	LC_{50}	LC_{100}
Glossiphonia complanata	15-25	15	80	300
Helobdella stagnalis	5-10	3	50	250
Caspiobdella fadejevi	15-25	0.003	0.07	0.5
Piscicola geometra	20-35	0.01	0.8	1.5
Hirudo medicinalis	80	0.05	0.3	0.6
Haemopis sanguisuga	70-90	2.5	10	25
Herpobdella octoculata	30-45	0.5	1.5	1.5
H. testacea	30-45	0.1	0.6	1
H. nigricollis	25-35	0.03	0.2	0.8
H. (*Dina*) *lineata*	20-40	1	3.5	8
Crustaceans				
Streptocephalus torvicornis	Adult	--	0.04	--
Daphnia pulex	Adult	0.00007	0.00028	0.00141
Asellus aquaticus	Adult	--	0.4	--
Mollusks				
Limnaea stagnalis	Adult	0.00515	0.5	51.5
Planorbis corneus	Adult	1	50	250
Carbofos				
Leeches				
Hirudo medicinalis	60-80	6.5	11	14
Herpobdella octoculata	30-45	4.5	6.5	8
H. nigricollis	20-30	1	4.7	7

TABLE 1. (Continued)

Pesticides and Organisms	Stage, size, mm	Maximum tolerated concentration (MTC)	LC_{50}	LC_{100}
Crustaceans				
Daphnia pulex	Adult	0.0001	0.0013	0.0177
Asellus aquaticus	Adult	0.011	0.27	8
Mollusks				
Limnaea stagnalis	Adult	11.2	36.7	163.2
Planorbis corneus	Adult	72.9	148.0	359.7
Rogor				
Leeches				
Hirudo medicinalis	60-80	2	28	40
Herpobdella octoculata	30-45	5	--	100
H. nigricollis	20-30	3	--	100

Roe had the lowest resistance from the time of fertilization to cleavage and at the organogenesis stage. Larvae on exogenous nutrition and fry at early stages of development also have low resistance (Guseva 1980). Among larvae and fry, the younger specimens perish faster (Table 4). For fry, 96-h LC_{50} of most agents constitutes 0.1-10 mg/ℓ (Johnson and Finley 1964, Post and Schroeder 1971, Grischenko et al. 1975, Prokopenko et al. 1975).

Current conceptions of the mechanisms of animal resistance to organophosphorus compounds are based on results obtained primarily for mammals and terrestrial anthropods (O'Brian 1971, Rozengart and Shestobitov 1978). It was established that resistance involved many factors, among which we can single out three basic mechanisms: difference in rate of penetration of toxicant into the body, metabolism of compounds in the body, and sensitivity of cholinesterases, which are the "targeted" enzymes. The last is the most important, because affinity of the enzyme for a toxicant determines the toxicity of organophosphorus pesticides.

In aquatic animals, as in mammals, cholinesterases are represented by two types: acetylcholinesterase (ACE) and cholinesterase (CE). The enzyme

TABLE 2. TOXICITY OF ORGANOPHOSPHORUS PESTICIDES FOR FISH WITH 48-h EXPOSURE AT TEMPERATURE OF 18-21°C, mg/ℓ

Fish species	Stage, age, and size, mm	MTC	LC_{50}	LC_{100}
Chlorofos				
Cyprinus carpio	Current Year Brood (CY)	90	340	1157
	2-year old	--	--	500
Carassius carassius	CY	--	--	150
	2-year old	--	100	--
Abramis brama	200	--	--	200
A. ballerus	200	--	--	70
Rutilus rutilus	140	--	30	60
Perca fluviatilis	120	0.25	0.62	1.95
Salmo irrideus	CY	--	1	--
Lebistes reticulatus	Adult	--	14	--
DDVP [dichlorvos]				
Cyprinus carpio	CY	12.1	21.9	44.1
Perca fluviatilis	CY	0.37	0.59	1
Carbofos				
Cyprinus carpio	CY	30	50	100
Perca fluviatilis	CY	--	0.034*	--

*According to Prokopenko et al. 1975

TABLE 3. SURVIVAL OF FISH LEECH COCOONS IN CHLOROFOS SOLUTIONS, 48-h EXPOSURE

Chlorofos concentration, mg/	Cocoon age, days	Number of cocoons	Hatched fry	
			quantity	%
Caspiobdella fadejevi				
(period of embryonic development, 20 days)				
20	1-4	33	3	9
20	10-11	40	0	0
20	18-20	27	0	0
10	1-4	44	44	100
10	9-11	50	18	36
10	17-20	30	2	6.7
2	1-4	84	84	100
2	9-12	50	36	70
2	18-20	31	15	48.5
Control	--	175	175	100
Pisciocola geometra				
(period of embryonic development, 14 days)				
50	1-2	24	10	41.5
50	7-8	25	0	0
50	10-12	41	0	0
5	3-4	44	44	100
5	7-8	48	28	58.5
5	11-12	35	13	37
Control	--	65	65	100

TABLE 4. TOXICITY OF ORGANOPHOSPHORUS COMPOUNDS FOR FISH AT DIFFERENT STAGES OF LIFE CYCLE, 96-H LC_{50}

Fish species	Stage, weight g	Malathion	Metathion	Benzophosphate	Source
Salmo clarki	Fry - 0.33	0.150	--	--	Post & Shroeder
	Fry - 1.25	0.201	--	--	
Salvelinus fontinalis	Fry - 1.15	0.120	--	--	Post & Shroeder
	Fry - 2.13	0.130	--	--	
S. namaycush	Fry - 0.3	0.76	--	--	Johnson & Finley
	Fry - 4.5	0.152	--	--	
Acipenser stellatus	Larvae	--	0.058**		Prokopenko et al.
	Fry	--	0.190**	--	
Cyprinus carpio	Larvae	--	--	0.008*	Prokopenko et al.
	Fry	--	--	0.074*	
	Fry	--	--	1.18	Grischenko et al.
	Current year, year-old	--	--	1.19	

*24-h LC_{50}
**Chronic LC_{50}

of the fish brain is typical ACE. There is ACE in blood serum of fish, the properties of which resemble the enzyme of the brain. At the same time, there is a blood serum enzyme referable to the CE type in some representatives of the family of Cyprinidae (zope, roach, bream) [10]. In the tested aquatic invertebrates (with the exception of the fresh-water oligochaetous worm, *Tubifex tubifex*), there is one enzyme of the ACE type represented in different molecular forms. Homogenates of the oligochaetous worm have high BuCE and PrCE activity, which warrants the belief that this species has two enzymes (ACE and CE), or one enzyme that occupies an intermediate position, between ACE and CE, with regard to its properties (Figures 1 and 2).

Data on inhibition of fish brain ACE by chlorofos, DDVP--metabolite of chlorofos and carbofos--are listed in Table 5. All of the tested species showed similar extent of inhibition of this enzyme by chlorofos. The values of pI_{50} constituted 3.6 for the enzyme of roach (*Rutilus rutilus*) and bream (*Abramis brama*), and about 4.0 for the enzyme of carp (*Cyprinus carpio*) and perch (*Perca fluviatilis*). The values of pI_{50} and K_{11} are higher with carbofos and DDVP than with chlorofos, which is indicative of the more marked inhibitory effect of these toxicants. We also failed to observe species-specific differences in enzyme sensitivity to DDVP and carbofos.

TABLE 5. VALUES OF pI_{50} AND K_{11} FOR FISH BRAIN ACETYLCHOLINESTERASE DURING INTERACTION WITH CHLOROFOS, DDVP, AND CARBOFOS IN VITRO

Fish species	Chlorofos pI_{50}	DDVP pI_{50}	DDVP K_{11}	Carbofos pI_{50}
Roach	3.6	--	--	--
Bream	3.6	--	--	--
Carp	4.2	5.6	$(9.2 \pm 0.33) \cdot 10^3$	5.0
Perch	4.0	5.4	$(5.6 \pm 0.14) \cdot 10^3$	4.5
Crucian carp	--	--	--	4.8

A comparison of sensitivity of ACE of aquatic invertebrates and fish to chlorofos established that in most invertebrates the enzyme is inhibited to a greater extent than in fish. The water flea (least resistant of invertebrates) and carp (most resistant of fish) present a 7000-fold difference in sensitivity of the enzyme. DDVP is more active for cholinesterases of both fish and invertebrates than chlorofos. Carbofos inhibits cholinesterases to a lesser extent (Table 6).

There also is a correlation between enzyme sensitivity and product toxicity in species that are close in their systematic place. Thus, in two species of gastropod mollusks, the large snail (*Limnaea stagnalis*) and the trumpet snail (*Planorbis corneus*), which differ by 100 times in resistance to chlorofos, an enzyme of the ACE type is present in nerve ganglia, which differs in relative electrophoretic mobility (REM) and sensitivity to the toxicant. In the less resistant species, the large snail, this enzyme is more inhibited by chlorofos (Figure 3).

In the species of the family Cyprinidae that we studied, brain and blood serum ACE had the same sensitivity to chlorofos. At the same time, there was a correlation between fish resistance to this toxic agent and blood serum CE sensitivity. For example, blood serum contains toxicant-sensitive CE in the fish species that is less resistant to chlorofos (zope, 48-h LC_{100} = 70 mg/ℓ), and the more resistant species (carp, 48-h LS_{100} = 500 mg/ℓ) does not have this enzyme (Figure 4).

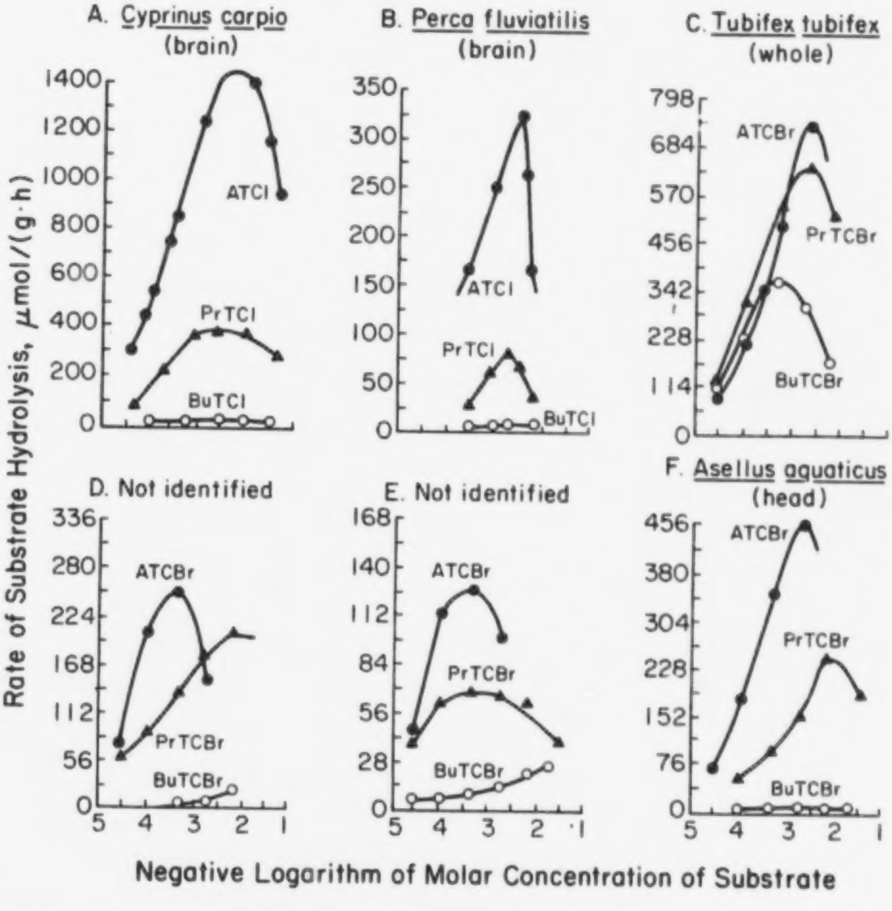


Figure 1. Substrate specificity of aquatic animal cholinesterases at 30°, pH 7.5.

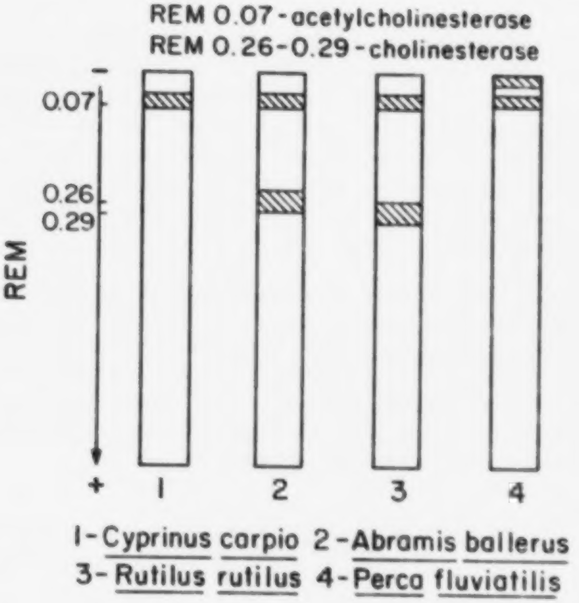

REM 0.07 - acetylcholinesterase
REM 0.26-0.29 - cholinesterase
−
0.07
0.26
0.29
REM
+
1
2
3
4
1- Cyprinus carpio 2 - Abramis ballerus
3- Rutilus rutilus 4- Perca fluviatilis

Figure 2. Electrophoregram of fish blood serum cholinesterases.

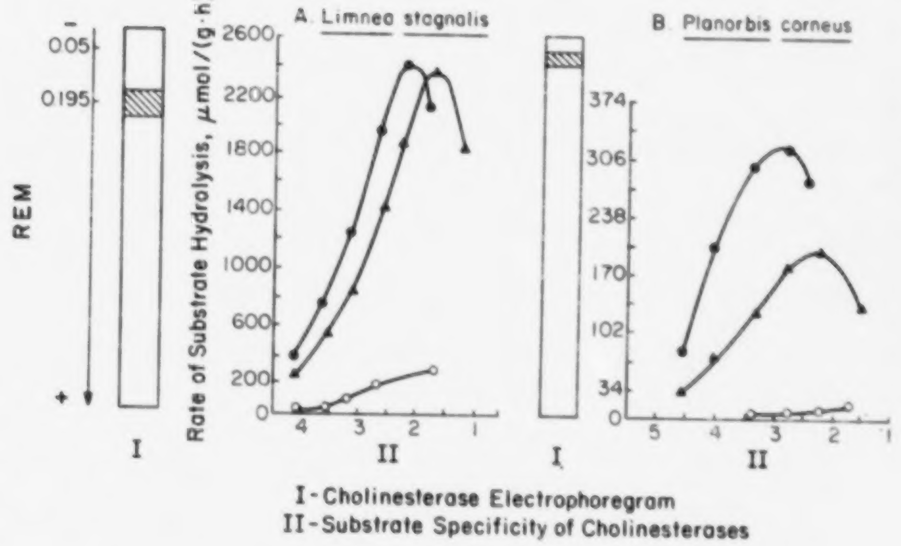


Figure 3. Acetylcholinesterase of mollusk ganglia.

TABLE 6. INHIBITION OF ACETYLCHOLINESTERASE OF SOME SPECIES OF AQUATIC INVERTEBRATES AND FISH IN VITRO BY CHLOROFOS, CARBOFOS AND DDVP, I_{50} (INHIBITOR CONCENTRATION ELICITING 50% DEPRESSION OF ENZYME ACTIVITY) IN 30 MIN.

Animal species	Source of enzyme	Chlorofos, M	DDVP M	Carbofos M
Daphnia magna	Whole body	$7.5 \cdot 10^{-8}$	--	--
Tubifex tubifex	Whole body	$9 \cdot 10^{-7}$	--	$5 \cdot 10^{-6}$
Limnaea stagnalis	Ganglia	$5 \cdot 10^{-6}$	$1.1 \cdot 10^{-7}$	$7 \cdot 10^{-5}$
Chironomus plumosus	Whole body	10^{-5}	$5.1 \cdot 10^{-6}$	$5 \cdot 10^{-5}$
Planorbis corneus	Ganglia	$5 \cdot 10^{-5}$	--	$5 \cdot 10^{-5}$
Asellus aquaticus	Head	$8 \cdot 10^{-5}$	--	--
Cyclops sp.	Whole body	$3 \cdot 10^{-4}$	--	$8 \cdot 10^{-4}$
Parca fluviatilis	Brain	10^{-4}	$4.1 \cdot 10^{-6}$	$2.9 \cdot 10^{-5}$
Abramis brama	Brain	$5 \cdot 10^{-4}$	--	--
Cyprinus carpio	Brain	10^{-4}	$2.5 \cdot 10^{-6}$	10^{-5}
Rutilus rutilus	Brain	$5 \cdot 10^{-4}$	--	--
Carassius carassius	Brain	--	--	$1.68 \cdot 10^{-5}$

A correlation between enzyme sensitivity to organophosphorus pesticides and systemic resistance is demonstrable in the same species with regard to different toxicants. Thus, *T. tubifex* and *L. stagnalis* are less resistant to chlorofos than carbofos, and the enzyme of these species is also more sensitive to chlorofos (Table 7).

Regardless of degree of ACE sensitivity to the toxic agents in vitro, a decline in enzyme activity occurs in the case of both acute and sublethal intoxication, and it precedes appearance of external signs of poisoning. Thus, in carp exposed to acutely lethal concentrations of carbofos (49-h LC_{50} = 50 mg/ℓ, in a state of heightened excitability, hydrolyzing capacity of brain ACE decreased by 54.7%, with 73.5% loss of equilibrium reflex. Thirty percent of the fish died within 24 h, and the rest were on their side at the bottom of the aquarium. In them, enzyme activity was very low--7.8% (Table 8).

With sublethal concentrations, upon appearance of the first signs of poisoning there were also reliable differences in ACE activity between the experiment and the control. When loss of the equilibrium reflex was observed, enzyme activity constituted only 15.5%. Thereafter, the condition of the fish improved. After 2 days there was recovery of the equilibrium reflex and ACE activity also rose somewhat, but on the whole, enzyme level was very low after both 2 and 5 days--19.5 and 22.2%, respectively. Enzyme activity was not fully restored at a later time (10-20 days), although outwardly the condition of the fish did not differ from the control (Table 9) (Kozlovskaya 1983).

In gastropod mollusks (L. stagnalis and P. corneus), the first symptom of chlorofos intoxication was weight gain due to excessive accummulation of fluid in the body. A comparison of changes of weight and ACE activity of nerve ganglia revealed that the decline in enzyme activity preceded the increase in mass (Table 10) (Kozlovskaya et al. 1982).

Hog slater (A. aquaticus) specimens placed in glass containers without rough surfaces are capable of aggregating (forming collections). In the presence of chlorofos poisoning, depending on its severity, the animals separate (crawl around the dish) due to their excited state. Reliable inhibition of ACE (by 45-50%) occurs after 4 h, with 0.01 mg/ℓ chlorofos concentration, which is distinct disaggregation.

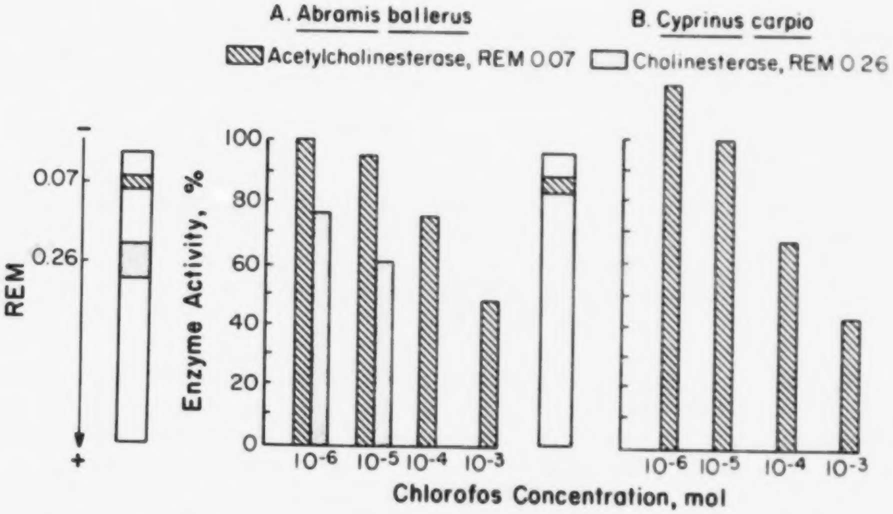


Figure 4. Fish blood serum cholinesterases and their sensitivity to chlorofos.

TABLE 7. TOXICITY OF CHLOROFOS AND CARBOFOS FOR TUBIFEX TUBIFEX AND LIMNAEA STAGNALIS, AND CHOLINESTERASE SENSITIVITY TO THESE AGENTS

Animal species	48-h LC_{50}, mg/ℓ		Enzyme inhibition, I_{50} in 30 min, M	
	chlorofos	carbofos	chlorofos	carbofos
T. tubifex	0.029	11.75	$9\cdot10^{-7}$	$5\cdot10^{-6}$
L. stagnalis	0.5	36.7	$5\cdot10^{-6}$	$7\cdot10^{-5}$

All of the foregoing warrants the conclusion that the resistance of aquatic animals to organophosphorus pesticides depends on sensitivity of their cholinesterases. However, one cannot fail to consider other factors as well. Resistance could be due to the rate of penetration of the toxic agent into the body and to detoxification processes, as indicated by the experiments dealing with toxicity of chlorofos and DDVP for fish as related to different routes of administration.

The carp and perch differ significantly in resistance to chlorofos and DDVP (Table 11). Investigation of sensitivity of brain ACE in the carp and perch to chlorofos revealed that pI_{50} values are similar. 4.0 for the carp and 4.2 for the perch. Brain and blood serum enzymes of these two species also fail to differ in sensitivity to DDVP. The K_{II} values found for them are of the same order, and are in the range of $5.6\cdot10^{3}$ to $9.2\cdot10^{3}$ m^{-1} min^{-1}.

TABLE 8. CARP BRAIN ACE ACTIVITY IN THE PRESENCE OF ACUTE CARBOFOS POISONING, 48-H LC_{50} = 50 mg/ℓ

Dynamics of manifestation of poisoning symptoms	Number of fish	Acetylcholinesterase activity	
		μmol ATC/(g·h)	% of control
35 min--heightened excitability	10	168.9±39.5*	45.3
1.5 h--loss of equilibrium reflex	10	98.4±26.3*	26.5
24 h--loss of sensitivity	10	29.2±9.9*	7.8
24 h--deaths	10	45.3±11.0*	12.2
48 h--loss of sensitivity	10	24.3±4.07*	6.5

*Difference between control and experiment is reliable with p 0.001.

TABLE 9. CARP BRAIN ACETYLCHOLINESTERASE ACTIVITY WITH SUBLETHAL CONCENTRATION OF CARBOFOS, 30 mg/ℓ

Dynamics of manefestation of poisoning symptoms	Number of fish	Acetylcholinesterase activity	
		µmol ATC/(g·h)	% of control
1 h--heightened excitability	10	206.5±54.01*	55.4
1 day--loss of equilibrium reflex	10	57.8±15.02*	15.5
2 days--restored equilibrium reflex, but body is arched when fish swims	10	72.7±17.39*	19.5
5 days--normal fish behavior, but integument is dark	10	82.6±22.9*	22.2
10 days--condition does not differ from control fish	10	151.5±40.5*	40.6
20 days--condition does not differ from control fish	10	210.0±69.96*	56.4

*Difference between control and experiment is reliable with p 0.001.

With the intraperitoneal route of intoxication, the carp also is more resistant to DDVP than the perch. In this case, however, it is only 10 times more resistant than the perch (Table 12).

Intraperitoneal injection of this toxic agent precludes the route of its usual penetration into fish. For this reason, the higher value for the ratio of LC_{50} of DDVP for carp to LC_{50} for perch with the usual mode of intoxication, as compared to the LD_{50} ratio with intraperitoneal injection is indicative of faster penetration of the toxic agent into the perch. This warrants the conclusion that the rate of DDVP penetration determines the differences in fish resistance to it. Water and substances it contains penetrates into all fresh-water fish mainly through the gills. The skin is virtually uninvolved in this process (Prosser 1977, Schmidt-Nielson 1982).

The difference noted between DDVP resistance of carp and perch when given by intraperitoneal injection can apparently be attributed to the difference in rate of its detoxification in fish, which occurs enzymatically, mainly in the liver. Fish liver homogenates actively break down organophosphorus compounds, including DDVP (Hogan and Knowles 1968). It was shown that this process is faster in carp than in other fish species (Fujii and Asaka 1982).

TABLE 10. CHANGE IN WEIGHT AND CHOLINESTERASE ACTIVITY OF GANGLIA IN *LIMNAEA STAGNALIS* WITH CHLOROFOS INTOXICATION

Time of experiment, h	Number of animals	Average weight, %	Cholinesterase activity, %
0	15	100	100
3	15	100	62.3*
6	15	108.4	45.7*
24	15	126.3*	26.9*
48**	15	160.5*	15.2*
48***	12	97.1	9.1

*Difference between control and experiment is reliable with p 0.01.
**Live mollusks
***Dead mollusks

The information about toxicity of organophosphorus pesticides for aquatic animals makes it possible to assess, to some extent, their adverse effect on aquatic fauna. Thus, judging by the results of investigating acute toxicity, we should expect that the presence of pesticides in reservoirs would have a devastating effect on leeches of the Herpobdellidae and Ichthyobdellidae families and, among those in the Hirudinidae family, it would affect *Hirudo medicinalis*, which is useful in medicine, but would not have a deleterious effect on the predatory leech *Haemopis sanguisuga*. Among fish, representatives of the Salmonidae and Percidae families would be the most susceptible to organophosphorus pesticides. Fish would be less resistant to toxic agents at early stages of ontogenesis. Of all the animals studied, crustaceans would be the most vulnerable.

Cladocera and, in particular, *Daphnia pulex*, which have low resistance to organophosphorus compounds, are the most suitable as test objects to regulate levels of water pollution by organophosphorus compounds and assess the toxicity of sewage from industrial enterprises. One should bear in mind, however, that plankton crustaceans have low resistance to a number of pollutants. For this reason, in order to demonstrate expressly organophosphorus compounds in water it is necessary to have adequate methods of identifying them. The enzymatic method could be used with success for this purpose; it is based on measurement of cholinesterase activity in animals exposed to a toxic agent or with direct introduction of the enzyme into a toxic medium. Commercial preparations and homogenates of aquatic animal organs and tissues, which contain CE that is more sensitive to organophosphorus compounds than ACE, can serve as sources of enzyme for such purposes.

Because production and use of organophosphorus pesticides will remain high in the future, along with regulations for their use, it is important to search for highly selective agents, with consideration of their potential toxicity for aquatic animals. It is possible to solve the problem of synthesizing agents with selective action only if the mechanisms of resistance to organophosphorus compounds of animals in different systematic groups are known.

In aquatic animals, resistance to organophosphorus compounds is largely determined by the sensitivity of their target enzymes, ACE and CE. In aquatic invertebrates that have low resistance to organophosphorus compounds, ACE is more sensitive to toxic agents than in fish. Resistance of gastropod mollusks also depends on the senstiivity of ACE of their ganglia. Resistance of fish in the Cyprinidae family is unrelated to sensitivity of ACE of the brain, but is related to sensitivity of blood serum CE. As shown by the results of the experiments with the carp and perch, there may also be mechanisms other than sensitivity of target enzymes, such as rate of penetration of the toxic agent into the body and processes of its detoxification, that determine the resistance of fish to organophosphorus compounds.

TABLE 11. TOXICITY OF CHLOROFOS AND DDVP FOR PERCH (PERCA FLUVIATILIS) AND CARP (CYPRINUS CARPIO) WHEN FISH ARE PLACED IN TOXIC ENVIRONMENT

Fish species	Number of fish	Length of fish, mm M±m	48-h LC_{50}, mg/ℓ	Ratio of carp LC_{50} to perch LC_{50}
Chlorofos				
Carp	28	62±2	340.0 (269.8÷428.4)*	548
Perch	48	64±1	0.62 (0.55÷0.70)	
DDVP				
Carp	36	57±1	21.9 (20.2÷23.8)	37
Perch	42	63±1	0.59 (0.54÷0.64)	

*Confidence intervals of LC_{50}

TABLE 12. TOXICITY OF DDVP FOR PERCH (PERCA FLUVIATILIS) AND CARP (CYPRINUS CARPIO) WHEN GIVEN BY INTRAPERITONEAL INJECTION

Fish species	Number of fish	Length of fish, mm M±m	LD_{50}, µg/g	Ratio of carp LC_{50} to perch LC_{50}
Carp	20	109±2	292.0 (254.0÷336.0)*	9.6
Perch	42	109±2	30.4 (23.0÷40.1)	

*Confidence interval of LD_{50}

BIBLIOGRAPHY

Chuyko, G.M., V.I. Kozlovskaya, and V.M. Stepanova. 1983. Blood serum carbonate esterases in the zope (Abramis ballerus), roach (Rutilus rutilus), bream (Abramis brama) and perch (Perca fluviatilis). Manuscript filed with the All-Union Institute of Scientific and Technical Information, IBVV [Institute of Biology of Inland Waters], USSR Academy of Sciences, 22 February 1983, File No. 6193-83. 20 p.

Fujii, Y. and S. Asaka. 1982. Metabolism of diazinon and diazoxon in fish liver preparations. Bull. Environ. Contam. Toxicol. 29(4). 455-460.

Grishchenko, L.I., A.P. Verkhovskiy, and G.A. Trondina. 1975. Toxicity of benzophosphate (Phosalone) for fish and detection of poisoning. In. Byulleten' Vsesoyuznogo instituta eksperimental'noy veterinarii (Bulletin of the All-Union Institute of Experimental Veterinary Science). Moscow, USSR. pp. 58-61.

Guseva, S.S. 1980. Effect of organophosphorus compounds on embryonic and early postembryonic development of the carp. Abstract of candidatorial dissertation--biological sciences. Moscow, USSR. 22 pages.

Hogan, J.W. and C.O. Knowles. 1968. Degradation of organophosphates by fish liver phosphatases. J. Fish Res. Board Canada. 25(8).1571-1579.

Johnson, W.W. and M.T. Finley. 1964. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates No. 137. Washington, USA. 98 p.

Kozlovskaya, V.I., T.V. Volkova, and V.T. Komov. 1982. Cholinesterase of neural ganglia and water metabolism in Limnaea stagnalis in the presence of chlorofos poisoning. In. Biologiya vnutrennikh vod. Inform. byul. (Biology of Inland Waters. Information Bulletin) No. 55. Leningrad, USSR. pp. 49-51.

Kozlovskaya, V.I., V.M. Stepanova, and G.M. Chuyko. 1983. Reversibility of carbofos poisoning of carp. In: Reaktsii gidrobiontov na zagryazneniye (Hydrobiont Reactions to Pollution). Leningrad, USSR. pp. 191-198.

Lapkina, L.N. 1983. Leeches in the Rybinskiy Reservoir and their resistance to toxic agents. Abstract of candidatorial dissertation--biological sciences. Moscow, USSR. 23 p.

Lapkina, L.N. and B.A. Flerov. 1979. Investigation of acute poisoning by certain toxic agents of leeches. In: Fiziologiya i parazitologiya presnovodnykh zhivotnykh (Physiology and Parasitology of Fresh-Water Animals). Leningrad, USSR. pp. 50-59.

O'Brian, R. 1971. Toxic phosphates. Moscow, USSR. 631 p.

Perevoznikov, M.S. 1979. Ichthyocidal properties of carbofos. GosNIORKh No. 146. Leningrad, USSR. pp. 42-52.

Post, G. and T.R. Schroeder. 1971. The toxicity of four insecticides to four salmonid species. Bull. Environ. Contam. Toxicology. 6(2): 144-145.

Prokopenko, V.A., N.P. Sokol'skaya, S.S. Nikulina, and N.R. Kosinova. 1975. Comparative ichthyotoxicological characteristics of methylnitrophos and methathion. In. Problemy vodnoy toksikologii (Problems of Marine Toxicology) Vol. 1. Petrozavodsk, USSR. pp. 110-112.

Prosser, L. 1977. Fluid metabolism: Osmotic balance and hormonal regulation. In: Sravnitel'naya fiziologiya zhivotnykh (Comparative Animal Physiology) Vol. 1. Moscow, USSR. pp. 27-176.

Rozengart, V.I. and O.Ye. Shestobitov. 1978. Izbiratel'naya toksichnost' fosfororganicheskikh insektoakaritsidov (Selective Toxicity of Organophosphorus Insecticides-Acaricides). Leningrad, USSR. 173 p.

Schmidt-Nielsen, K. 1982. Animal physiology. Moscow, USSR. 800 p.

ACUTE TOXICITY OF IRON CYANIDES AND THIOCYANATE TO TROUT

by

R.V. Thurston[1]
T.A. Heming[2]

ABSTRACT

Toxic effects to rainbow trout and brook trout of exposure to ferrocyanide, ferricyanide, and thiocyanate were examined under controlled light conditions in a series of 96-hour laboratory tests. Solutions of both iron cyanides were more acutely toxic to rainbow trout when tested under light than when tested in total darkness. Thiocyanate was acutely toxic at concentrations lower than suggested in a previous study, but its effects appeared to be relatively unpredictable.

INTRODUCTION

The toxic effects of cyanides arise primarily from the formation of CN^- complexes with the metal ions present in many proteins and the subsequent inhibition of protein function (for review see Vennesland et al. 1981). In vertebrates, cytochrome c is the protein most sensitive to cyanide and, as a consequence, the principal effect of cyanide is to inhibit oxidative phosphorylation at the level of the mitochondria. The major pathway for detoxification of cyanide in most animals is via conversion of cyanide to thiocyanate (SCN^-), a reaction that is catalyzed by the enzyme rhodanase. The resultant thiocyanate is excreted via the urinary tract.

Acute and chronic toxic effects of cyanides, and the effect levels, have been extensively studied in most groups of animals including fishes (for review see Doudoroff 1976, Towill et al. 1978). Similar information regarding iron cyanides, thiocyanate, and cyanate is limited, however, especially for fishes and other lower vertebrates. This lack of data is especially apparent with respect to the characteristics and kinetics of uptake and excretion, tissue distribution, and metabolism.

---

[1] Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT 59717
[2] Aquatic Toxicology Section, Alberta Environmental Centre, Vegreville, Alberta, Canada T0B 4L0

Ferrocyanide [$Fe(CN)_6^{4-}$] and ferricyanide [$Fe(CN)_6^{3-}$] are byproducts of the cyanidation process employed in the extraction of gold and silver from ore. Both complexes are present frequently in ore mine effluents. Environmental concerns about these iron cyanide complexes generally center around their photodegradation to free cyanide. Light of wavelength less than 420 nm is active in the decomposition of ferrocyanide, and of less than 480 nm in the decomposition of ferricyanide (Broderius and Smith, 1980). Light source and intensity, therefore, are critical in any toxicity testing with these two anions. Free cyanide concentration, as well as total cyanide concentration, is important in interpreting test results. Although quality and intensity of light appear to be the most important factors in the decomposition process, temperature, pH, and dissolved oxygen also play significant roles.

Thiocyanate enters the aquatic biosphere directly as SCN^- and indirectly as a product of the metabolism of cyanide. The fact that endogenous conversion of cyanide to thiocyanate is the principal pathway for cyanide detoxification in many animals does not infer that thiocyanate is not toxic. Thiocyanate inhibits transport of halides in the thyroid (Wolff, 1964), stomach (Davenport 1940, Davies 1951), cornea (Zadunaisky et al. 1971), and gills (Epstein et al. 1973, 1975) and also inhibits various enzymes such as carbonic anhydrase (Davenport 1940), succinic dehydrogenase (Solomon et al. 1973), and moieties of ATPase (Katz and Epstein 1971, Solomon et al. 1973).

Thiocyanate has been used therapeutically in the treatment of hypertension in humans, occasionally with unpredictable and fatal consequences (Garvin 1939, Goodman and Gilman 1970). Toxic effects to humans are the result of an action of SCN^- on the central nervous system and include irritability, nervousness, hallucinations, psychosis, mania, delirium, and convulsions (Barnett et al. 1951).

Evidence of the conversion of inorganic and organic thiocyanates to cyanide has been found in mammals (Boxer and Rickards 1952a, 1952b, Goldstein and Reiders 1951, Pines and Crymble 1952). It has been proposed that these conversions involve a red cell enzyme, thiocyanate oxidase (Goldstein and Reiders 1953), and the enzyme glutathione-S-transferase (Habig et al. 1975).

Information on the toxicity of thiocyanate to aquatic life forms is scant and contains conflicting results (for reviews see Doudoroff 1976, Huiatt et al. 1983, Towill et al. 1978). Doudoroff (1976), for example, noted effect levels under various conditions to occur at concentrations ranging from 29 to 5000 mg/liter SCN^-; he concluded that "thiocyanate is somewhat toxic." APHA et al. (1980) have dismissed the toxicity of thiocyanate as being relatively unimportant unless subsequent chlorination, which would produce cyanogen chloride, is anticipated. The U.S. Environmental Protection Agency (U.S. EPA 1980) does not list a water quality criterion for thiocyanate. Further examination of the aquatic toxicity of thiocyanate is timely because of the recent development of the INCO SO_2/air process for cyanide removal from industrial effluents. This process selectively oxidizes cyanide and metal cyanide complexes, but has little effect on thiocyanate (Devuyst et al. 1982, Huiatt et al. 1983).

In this presentation, we will discuss the results of recent unpublished research at Fisheries Bioassay Laboratory (FBL) on the acute toxicity to fishes of both iron cyanide complexes and thiocyanate. The toxicants we tested were reagent grade potassium ferrocyanide, potassium ferrocyanate, and potassium thiocyanate. The test fish were hatchery-reared rainbow trout (Salmo gairdneri) and brook trout (Salvelinus fontinalis). Chemical analyses were conducted according to methods prescribed by APHA et al. (1980), ASTM (1981), and U.S. EPA (1974). Fish were tested using diluters either of the design of Benoit (1982) or Mount and Brungs (1967). Each diluter delivered water to one control tank and five test tanks; each of the test tanks was maintained at a different concentration of toxicant. Except as noted, ten fish were tested in each tank. Median lethal concentration (LC50) values were determined according to the Trimmed SpearmanKarber method (Hamilton et al. 1977). More complete details of the testing methods have been, or will be, reported elsewhere (Meyn et al. 1984, Heming et al. Submitted).

FERROCYANIDE AND FERRICYANIDE TOXICITY TESTS

TEST PROCEDURES

A series of 96-hour, flow-through toxicity tests was conducted on rainbow trout to determine the toxicity of ferrocyanide and ferricyanide under two light regimes: total darkness and a cycle of 18 hours light/6 hours darkness. A minimum of two tests were run with each chemical under each light regime. Four additional tests, two each on ferrocyanide and ferricyanide, were conducted at an elevated temperature under a cyclic regime of 18 hours light/6 hours darkness. The tanks were glass aquaria with glass covers, having a water volume of approximately 14 liters. The diluters had a flow rate of 30 ml/minute and the turnover time was approximately 8 hours. Tests were started with the test concentrations already established in the tanks. Fish for a given test were from a single pool and were distributed at random among the control and test tanks. The tests were conducted in a room equipped with wide-spectrum bulbs (Spectralite, Long-Life Lighting Products) that were reported to produce a spectral energy distribution similar to daylight. For tests conducted in total darkness, a red bulb was used for illumination during the collection of water samples and during mortality checks.

In designing the experiment, it was assumed that there might be marked differences in photodecomposition of the toxicants between a test regime of total darkness and one that provided light during a simulated daytime period. It was further assumed that, at relatively low light intensities (as compared with the intensity of bright sunlight, modest differences in intensity between tanks would not significantly affect test results. As a consequence, no special attempt was made to ensure that light intensities at the water surface of the different tanks in each test were identical. Two sets of test tanks were used; the range of light intensity at the tank water surface for one of these was 230 to 400 lux (mean 330 lux) and for the other was 550 to 780 lux (mean 680 lux). It subsequently developed that these light intensity differences most probably played a far greater role in determining the test results than originally anticipated; these differences may have been sufficient to mask differences in results attributable to temperature within the range tested (9 to 16°C).

Water samples for cyanide analysis were collected from the effluent of each tank three times during each test. Sodium hydroxide was added to each sample to raise the pH to 12; samples were then refrigerated and shipped to Homestake Mining Company at Lead, South Dakota, for analysis. Total cyanide (total CN^-) was determined using a modified acid reflux/distillation method (ASTM 1981), and "weak acid dissociable cyanides" were determined by "Method C" (ASTM 1982). Under our test conditions using FBL water, which contained only insignificant quantities of metals, "weak acid dissociable cyanides" was essentially free cyanide. Results for all tests were reported in terms of total CN^-; results for two tests in the dark and all tests in the light also were reported in terms of Method-C CN^-. All other water measurements were made at FBL, and included dissolved oxygen, temperature, pH, alkalinity, and hardness (Meyn et al. 1984).

It should be noted that solutions of ferricyanide ion are distinctly yellow. The intensity of color increases with increasing concentrations and was almost orange at the highest concentrations tested. By contrast, ferrocyanide ion imparts only a pale yellow color to its solutions, even at very high concentrations. The presence of iron interfered with alkalinity and hardness determinations in all but the control tanks. Light intensity measurements were made with a Gossen Panlux Electronic FootLambert meter, recorded as foot-candles and converted to lux.

The LC50 values derived in terms of either total CN^- or Method-C CN^- should be considered valid only under the conditions of testing, i.e., total darkness or 18-hour day, and at the specified temperature and concentration of dissolved oxygen. The LC50 values expressed in terms of Method-C CN^- are further restricted by being computed from the means of concentrations that increased during some of the tests.

TEST RESULTS

Comparison of the results of the ferrocyanide tests conducted in total darkness (Tests 1074, 1077, 1078) and those under an intermittent light regime of either 330 lux (Tests 1088, 1090) or 680 lux (Tests 1080, 1083) shows the fish were more sensitive to the test water in those tests conducted under light (Table 1). Comparison of results of the tests conducted under light further shows that the fish were more sensitive to the test water at 680 lux than at 330 lux. A variable introduced

TABLE 1. FERROCYANIDE TOXICITY TESTS - SUMMARY OF TEST CONDITIONS AND 96-HOUR LC50 VALUES OBTAINED (Ten fish per tank)

Test no.	Light regime, hours of light/dark	Mean light intensity, lux	Mean pH (range)	Mean temperature, °C (range)	Mean D.O., mg/liter (range)	Method-C CN^- 96-h LC50, mg/liter (95% C.I.)	Total CN^- 96-h LC50, mg/liter (95% C.I.)
1074	0/24	0	-	9.4 (9.4-9.5)	8.44 (7.03-8.85)	-	752 (474-1190)
1077	0/24	0	-	-	-	-	> 867
1078	0/24	0	7.95 (7.89-8.01)	9.4 (9.4-9.5)	8.83 (8.76-8.97)	1.10 (0.99-1.23)	939 (828-1064)
1088	18/6	330	7.77 (7.66-7.94)	15.7 (15.4-16.1)	8.22 (8.01-8.49)	0.79 (0.63-1.0)	220 (184-261)
1090	18/6	330	7.83 (7.75-7.95)	15.2 (15.2-15.4)	7.85 (7.64-8.11)	0.71 (0.51-0.98)	232 (178-302)
1080	18/6	680	7.33 (7.30-7.34)	9.6 (9.6-9.7)	7.93 (7.77-8.17)	0.15 (0.13-0.17)	33.0 (28.7-37.9
1083	18/6	680	7.55 (7.50-7.57)	9.5 (9.5-9.6)	8.37 (8.16-8.58)	0.17 (-)*	37.4 (-)*

*Confidence interval not calculable by method used.

in this second comparison, however, is that the 330-lux tests were conducted at a higher temperature (15 to 16°C) than those at 680 lux (9 to 10°C).

Comparison of the results of the ferricyanide tests conducted in total darkness (Tests 1075, 1076, 1079) with those conducted under an intermittent light regime of 680 lux (Tests 1085, 1087, 1089) shows markedly greater toxicity under light (Table 2). Of the three tests conducted at 680 lux, one was conducted at 10°C (Test 1085) and the other two at 16°C (Tests 1087, 1089). Results show a slight increase in toxicity at the higher temperature, but the overlap in LC50 confidence intervals for these tests suggests that the finding is not conclusive.

To see whether there are additional conclusions that might be drawn from these data, we combined the results of all tests and made the assumption that differences in toxicity, if any, resulting from temperature differences were over-ridden by other factors. We looked at all the data for each test, and from each we selected the tank with the highest Method-C CN^- concentration at which no more than 10% mortality occurred (Table 3). For two of the ferricyanide tests, more than 10% of the fish died at even the lowest concentration tested. These were Test 1087 with 40% mortality in Tank 2, and Test 1089 with 30% mortality in Tank 2. To include these tests in our data pool it was necessary to use these tanks with > 10% mortality, presumably ≤ 10% mortality would have occurred at a lower Method-C CN^- concentration. We then plotted the measured light intensity for each of these test tanks versus its measured Method-C CN^- concentration, using the highest single measurement of Method-C CN^- in each tank as opposed to the mean of three measurements. The rationale for selecting the highest measured Method-C CN^- value was that the toxic action of cyanide is rather rapid, and the fish that died were probably most affected by that highest concentration, rather than by a 96-hour average.

The results of this plot (Figure 1) are revealing. The estimated curves for both sets of tests (ferrocyanide and ferricyanide) are similar, and one may conjecture that there may not be a significant difference between them. The implications are that the toxicity of Method-C CN^- is light dependent, i.e., there is a correlation between the toxicity of Method-C CN^- and light intensity.

The behaviour and distress symptoms of fish in the ferrocyanide and ferricyanide acute toxicity tests were similar to, but less dramatic than, those that we also observed during acute toxicity tests on thiocyanate. These symptoms included flaring of the gills, gaping of the mouth, erratic and sudden movement, and immediate stiffness of the body when the fish died. The symptoms were particularly noticeable at the termination of a test when all remaining fish were anesthetized with tricainemethanesulfonate prior to weighing and measuring. The degree to which these symptoms were evident was directly related to toxicant concentration; at the lowest concentrations tested, and in the control tanks, fish did not evidence these symptoms.

TABLE 2. FERRICYANIDE TOXICITY TESTS - SUMMARY OF TEST CONDITIONS AND 96-HOUR LC50 VALUES OBTAINED (Ten fish per tank)

Test no.	Light regime, hours of light/dark	Mean light intensity, lux	Mean pH (range)	Mean temperature, °C (range)	Mean D.O., mg/liter (range)	Method-C CN^- 96-h LC50, mg/liter (95% C.I.)	Total CN^- 96-h LC50, mg/liter (95% C.I.)
1075	0/24	0	7.87 (7.46-8.23)	9.3 (9.1-9.4)	8.18 (7.03-8.85)	-	1210 (1060-1380)
1076	0/24	0	7.96 (7.75-8.17)	9.3 (9.2-9.4)	8.88 (8.70-9.01)	-	869 (680-1110)
1079	0/24	0	-	-	-	> 1.14	> 877
1081	18/6	330	7.33 (7.30-7.34)	9.6 (9.5-9.7)	7.91 (7.61-8.11)	0.24 (0.23-0.26)	69.6 (54.7-88.5)
1082	18/6	330	7.56 (7.53-7.58)	9.6 (9.5-9.7)	8.28 (8.09-8.43)	> 0.50	> 541
1084	18/6	330	-	-	-	> 0.61	> 731
1085	18/6	680	7.78 (7.76-7.80)	10.3 (10.1-10.7)	8.26 (8.13-8.34)	0.42 (0.36-0.50)	44.2 (38.2-51.0)
1087	18/6	680	7.96 (7.89-8.06)	16.0 (15.8-16.1)	8.69 (8.58-8.83)	0.40 (0.36-0.43)	38.8 (25.0-60.2)
1089	18/6	680	7.89 (7.79-8.10)	16.0 (15.5-16.4)	8.16 (8.05-8.37)	0.31 (0.30-0.32)	10.8 (8.96-13.1)

TABLE 3. LIGHT INTENSITY IN TANKS WITH HIGHEST METHOD-C CN^- CONCENTRATION AT WHICH NO GREATER THAN 10% MORTALITY OCCURRED

Chemical and test no.	Tank with ≤ 10% mortality: Highest measured Method-C CN^-, mg/liter	Light intensity, lux
Ferrocyanide		
1074	1.15	0
1078	1.13	0
1080	0.13	780
1083	0.14	590
1088	0.35	250
1090	0.49	310
Ferricyanide		
1079	1.30	0
1081	0.22	310
1082	0.64	400
1084	0.58	390
1085	0.46	780
1087	0.47*	780
1089	0.33†	780

*Test tank reported is that with lowest mortality (40%). [See text]
†Test tank reported is that with lowest mortality (30%). [See text]

THIOCYANATE TOXICITY TESTS

TEST PROCEDURES

Acute (96-hour) toxicity tests were conducted on both brook trout and rainbow trout under continuous-flow conditions as described above, except that these tests were started at zero toxicant concentration, and full test toxicant concentrations were effectively reached within 18 hours Tests were conducted under two conditions: an ambient photoperiod with fish visually exposed to normal laboratory traffic and activities and controlled photoperiods with fish isolated from these activities by curtains. Controlled photoperiods were 24-hours white light (223 lux), 12-hours light/12-hours dark, and 24-hours dim red light (0.2 lux). Effects of stress on thiocyanate toxicity were assessed in tests in which fish were forced to swim vigorously for 30 seconds, by inserting a hand-held dip net into the tank water and moving it around taking care not to touch the fish.

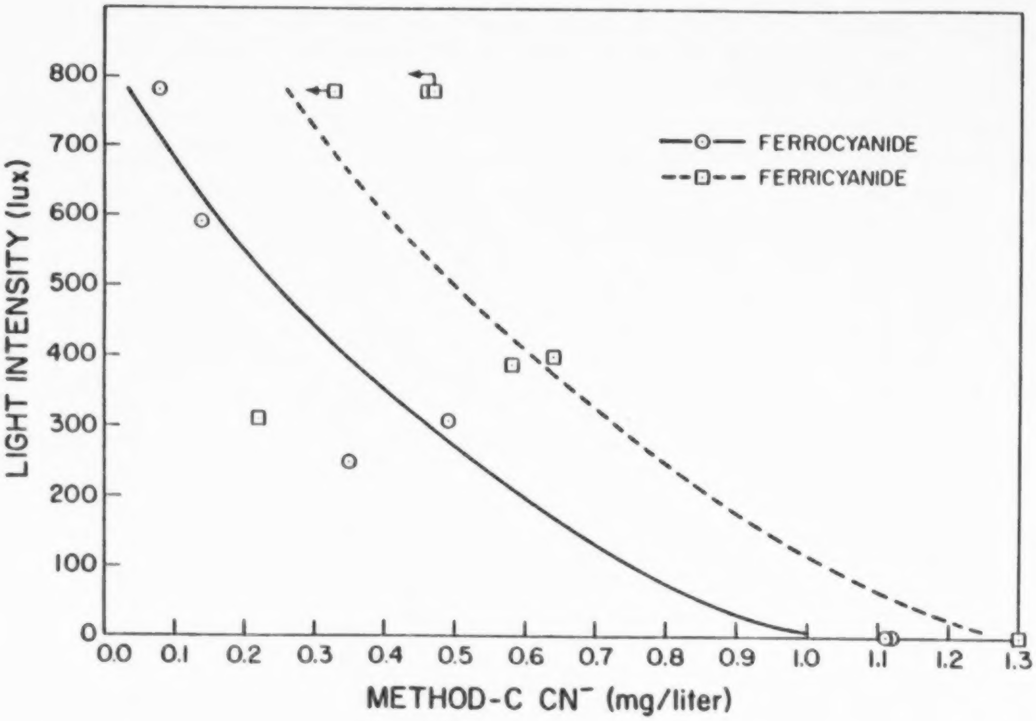


Figure 1. Test tank concentrations with ≤ 10% mortality vs. light intensity. (Ferricyanide data points with arrows indicate > 10% mortality.)

TEST RESULTS

In conducting our initial acute toxicity tests on brook trout, we observed that, although there was an overall dose response correlation, some fish survived the 96-hour test period at thiocyanate concentrations an order of magnitude greater than those that caused a high percentage of mortality to others (Table 4). We also observed that the death of fish frequently would be sudden, i.e. fish that evidenced little or no sign of distress at the time of routine scheduled mortality observations were under distress or dead shortly thereafter. We further observed that fish with relatively placid behaviour in a given tank markedly increased their swimming activity when we were collecting water samples, or when we were removing the fish from the tank at the end of the test; some of the seemingly healthy fish died in the dip net during removal from the tank. These mortalities in the tank or upon removal were characterized by sudden rapid movement, onset of tonic convulsions, loss of equilibrium and buoyancy, gasping, flaring of the operculae, darkening of the skin epithelia, and finally cessation of ventilation and extreme rigor. We termed this occurrence "sudden death syndrome" (SDS).

TABLE 4. PERCENT MORTALITY OF BROOK TROUT* IN EACH TEST TANK DURING 96-HOUR ACUTE TOXICITY TESTS ON THIOCYANATE† (Tests 651, 652, 653, 655)

Test no. - Tank no.	Exposure concentration, mg/liter SCN^-	No. of fish	Percent mortality at 96 h
651-6	517	10	90
651-5	375	10	90
651-4	298	10	80
651-3	232	10	80
652-6	198	10	90
651-2	163	10	80
652-5	148	10	70
652-4	115	10	90
653-6	96.4	15	73
652-3	90.8	10	90
653-5	72.1	15	47
652-2	65.4	9	56
653-4	56.1	15	73
653-3	44.3	15	53
653-2	31.6	15	47
655-6	31.3	10	50
655-5	24.1	10	70
655-4	18.5	10	30
655-3	14.8	10	20
655-2	10.5	10	0
All tests-1	0.0	45	0

*Mean fish size (and range): 6.7(5.56-7.94) g, 8.2(6.1-10.3) cm.

†Other measured water variables, mean values and ranges for all tanks: temperature 15.1(12.9-17.3)°C; dissolved oxygen 7.70(6.38-8.31) mg/liter; pH 7.88(7.83-7.90); alkalinity 176(167-181) mg/liter as $CaCO_3$; hardness 203(195-208) mg/liter as $CaCO_3$.

To test the hypothesis that SDS was related to the fish being distressed by activity near the test area, two 96-hour acute toxicity tests, one each on brook trout (Test 658) and rainbow trout (Test 723), were conducted during which activity near the test area was reduced to a necessary minimum. At the close of the standard 96-hour test periods, the fish were stressed with a dip net, and mortality counts taken 15 minutes later (Test 658) and 75 minutes later (Test 723) showed a dramatic increase over those taken at 96 hours (Table 5). Two additional 96-hour tests on brook trout (Tests 657 and 659) were conducted during which the fish (10 fish per tank) were stressed with a dip net at 24, 48, 72, and 95 hours. Fish size and measured water chemistry variables for these tests were similar to those reported for Test 658. The 96-hour LC50 for Test 657 was 13.8 mg/liter SCN$^-$ and that for Test 659 was 13.3 mg/liter SCN$^-$.

To determine whether the toxicity of thiocyanate might differ with different photoperiods, duplicate 96-hour tests were conducted on rainbow trout under each of three different light regimes: continuous exposure under red light, a daily regime of 12 hours white light and 12 hours in the dark, and continuous exposure to white light. The fish were least sensitive to thiocyanate toxicity under red light, more so under the alternate light/dark regime, and most sensitive under continuous exposure to white light (Table 6). At the end of the 96-hour test periods, the fish tested under continuous red light were briefly exposed to white light, and the light was briefly turned off on the fish tested continuously under white light; mortality counts were taken again 45 minutes later for each of these tests. Although not as dramatic as in the tests with trout subjected to dip net stress at the close of the 96-hour test period (Table 5), there was an

TABLE 5. PERCENT MORTALITY BEFORE AND AFTER STRESS OF FISH EXPOSED TO THIOCYANATE* (Ten fish/tank)

Tank no.	Brook trout† (Test 658) Exposure concentration mg/liter SCN$^-$	Brook trout† (Test 658) Percent mortality 96h	Brook trout† (Test 658) Percent mortality 96.25h	Rainbow trout** (Test 723) Exposure concentration mg/liter SCN$^-$	Rainbow trout** (Test 723) Percent mortality 96h	Rainbow trout** (Test 723) Percent mortality 97.25h
1	0.00	0	0	0.02	0	0
2	5.52	0	10	7.70	0	100
3	8.00	10	70	9.90	0	100
4	9.99	10	70	11.8	30	80
5	13.0	0	80	15.3	0	100
6	16.7	10	100	20.8	50	90

*Other measured water chemistry variables, mean values, and ranges for all tanks: Test 658 - temperature 16.3 (15.9-16.7)°C, dissolved oxygen 7.54(7.20-7.93) mg/liter, pH 7.86(7.83-7.95), alkalinity 164 mg/liter as $CaCO_3$ (all measurements), hardness 194(194-195) mg/liter as $CaCO_3$; Test 723 - temperature 11.8(11.3-12.5)°C, dissolved oxygen 9.35(9.20-9.78) mg/liter, pH 7.95(7.92-8.00), alkalinity 175(172-178) mg/liter as $CaCO_3$, hardness 202(198-206) mg/liter as $CaCO_3$.

†Mean fish size (and range) 8.9 g, 9.1(7.8-10.7) cm; 96-h LC50 > 16.7 mg/liter SCN$^-$, 96.25-h LC50 7.79 mg/liter SCN$^-$ (95% C.I. 6.62-9.16).

**Mean fish size (and range) 0.72(0.32-1.42) g, 4.16(3.15-5.25) cm; 96-h LC-50 20.8 mg/liter SCN$^-$ (95% C.I. not calculable by method used), 97.25-h LC50 < 7.70 mg/liter SCN$^-$.

TABLE 6. ACUTE TOXICITY OF THIOCYANATE TO RAINBOW TROUT* DURING TESTS† WITH DIFFERENT LIGHT REGIMES (Ten fish/tank)

Test no.	Light regime	LC50 mg/liter SCN^- (95% C.I.) 96-h	96.75-h
721	Continuous red light	159 (119-213)	76.3 (48.7-120)
725	Continuous red light	64.6 (13.2-316)	64.6 (13.2-316)
727	12h white light/12h dark	83.5 **	
728	12h white light/12h dark	55.7 (39.0-79.6)	
720	Continuous white light	48.3 (34.6-67.5)	38.7 (25.7-58.3)
724	Continuous white light	44.9 (30.0-67.2)	41.4 (27.4-62.5)

*Mean fish size (and range) 0.83(0.28-1.79) g, 4.42(3.10-5.55) cm.

†Other measured water variables, mean values, and ranges for all tanks: temperature 13.0(12.2-13.5)°C, dissolved oxygen 8.96(8.66-9.30) mg/liter, pH 7.93 (7.77-8.87), alkalinity 174(168-206) mg/liter as $CaCO_3$, hardness 202(200-206) mg/liter as $CaCO_3$.

**Confidence intervals not calculable by method used.

increase in mortality in three out of four of these tests following the sudden change in light regime.

The persistence of SDS was tested by stressing (dip net method) separate groups of juvenile rainbow trout (10 fish per group) at 0, 4, 12, 24, and 48 hours after exposure to thiocyanate for 96 hours under flow-through conditions, a single control group was stressed at each of these time periods. After the 96-hour exposure period, thiocyanate addition to the incoming water to each test tank was discontinued, concentrations in the test tanks at 4 hours were approximately 4 mg/liter SCN^-, and < 1 mg/liter SCN^- by 12 hours. The group stressed at 4 hours suffered 60% mortality, and none of the fish died after stress at 48 hours (Table 7).

DISCUSSION

Solutions of both ferrocyanide and ferricyanide proved to be more acutely toxic to rainbow trout when tested under light than when tested in total darkness. Further, there was a trend toward greater toxicity of either of these solutions at greater light intensity. Both of these potential toxicants are known to decompose under light to produce free cyanide. Although it was not possible for us to measure free cyanide, the concentration of Method-C CN^- was assumed to approximate the concentration of free cyanide.

If free cyanide alone were the toxic agent, and if Method-C CN^- concentrations approximate free cyanide concentrations, then calculation of LC50 values in terms of Method-C CN^- should have been comparable for all tests regardless of light regime. This was not the case; toxicity of the solutions tested in terms of Method-C CN^-, whether considering the LC50 values for either or both sets of tests, or considering the individual tanks with $\leq$ 10% mortality for both sets of tests combined, revealed a relationship between available light and mortality of fish.

We conclude that Method-C CN^- is not a true (approximate) measure of free cyanide under the conditions of our tests and/or that free cyanide was not the sole toxic agent in the tests conducted. Additional research will be necessary to quantify better the toxicity of solutions of iron cyanide complexes, and the conditions of their photodegradation. In conducting this additional research, greater care should be exercised to standardize light conditions both among tanks for any given test, and between tests that are to be compared for other variables such as temperature. Further, the procedures for analysis of different forms of cyanide in the test waters should be reviewed to ensure that samples collected have not appreciably altered by the time of analysis.

TABLE 7. PERSISTENCE OF SUDDEN DEATH SYNDROME IN JUVENILE RAINBOW TROUT* MAINTAINED IN FRESH WATER FOR DIFFERENT TIME PERIODS AFTER 96-HOUR EXPOSURE TO SCN^- (Test 726)

Group no.	Exposure concentration, mg/liter SCN^-	Mortalities during 96-h exposure	Hours in replacement water after SCN^- exposure and before 30s stress†	Additional mortalities within 45-m after stress	Total mortalities during entire test period
1	0.0	0	0	0	0
2	6.7	2	0	6	8
3	7.3	0	4	6	6
4	6.9	1	12	2	3
5	8.0	1	24	3	4
6	7.7	1	48	0	1

*Ten fish per group, mean size and range 1.03(0.37-2.48) g, 4.57(3.25-6.00) cm.

†No fish died during these periods.

The results of our tests on thiocyanate demonstrate it to be acutely toxic to trout at concentrations lower than those suggested by previous studies (see Doudoroff 1976). The acute toxicity of thiocyanate to trout, however, would appear to be relatively unpredictable. Exposed fish, which seemed healthy, would suddenly and often for no apparent reason begin to convulse and quickly expire.

The signs of SDS in trout (convulsions, loss of equilibrium and buoyancy, extreme rigor, among others) are virtually identical to the characteristics of acute thiocyanate toxicity in mammals, including humans (convulsions, vertigo, coma, hyper-reactivity, extensor rigidity) (Garvin 1939, Smith 1973). In mammals, these effects are probably due to the SCN^- ion itself, rather than to its metabolic products, HCN, OCN^-, and NH_3 (Smith 1973); administration of sodium thiosulphate, an antidote for cyanide poisoning, has no effect on thiocyanate toxicity in mammals. Thiocyanate toxicity in mammals would appear to be influenced by neural activity, inasmuch as sympathomimetic stimulants (e.g. amphetamines) increase the toxicity of SCN^-, whereas barbiturate anaesthetics (e.g. phenobarbital) decrease its toxicity (Smith 1973).

SDS in fish may represent a direct effect of the thiocyanate anion on neuromuscular functioning; SCN^- has been shown to enhance the contractility and electrical conduction of frog muscle fibers (Lubin 1957), and to increase the excitability and electrical transmission of spinal neural pathways and motoneurons (Goto and Esplin 1961). Regardless of the exact mechanism involved in SDS, normal rapid movements of wild fish during feeding and predator avoidance probably would be sufficient to trigger SDS as a consequence of thiocyanate exposure.

Standard acute toxicity tests (96-hour LC50 determinations) are of limited value in predicting the toxicity of thiocyanate because of the unpredictability of SDS. Subsequent to the studies reported here, we determined that the correlation between plasma thiocyanate concentrations and SDS provides a more certain measure of thiocyanate toxicity (Heming et al. Submitted). Those results demonstrated that fish readily accumulate SCN^- and 50% of the fish tested would be at risk of SDS when plasma SCN^- concentrations reached approximately 4 mmol/liter. The duration of thiocyanate exposure necessary to attain that plasma concentration depends upon the exposure concentration, fish size, water temperature, and ambient water quality.

It is possible that, under conditions when uptake of SCN^- is extremely slow, excretion of SCN^- would be able to keep pace with SCN^- uptake and plasma concentrations of thiocyanate would remain low. Under such circumstances, SDS would not be expected to occur. None of our experiments was run at a sufficiently low concentration of thiocyanate to test this hypothesis; the lowest concentration of toxicant we tested was 17 umol/liter KSCN. At that level of exposure, 6.7 g rainbow trout accumulated thiocyanate at a rate of approximately 15 mmol/liter plasma/day/kg fish. Given such a rate of SCN^- accumulation, one would expect that 50% of the exposed population would be at risk of SDS within 1-2 months. During such long term exposures, fish probably would encounter the chronic toxic effects of thiocyanate. In mammals, and presumably in fishes as well, SCN^- inhibits incorporation of I^- by the thyroid gland, which in the long term results in goiter (Wolff 1964).

ACKNOWLEDGMENTS

Elizabeth L. Meyn, Donald R. Skaar, and Richard K. Zajdel were principally responsible for performing the acute toxicity tests, and Ms. Meyn and Terry L. Mudder (Homestake Mining Company) for the water chemistry analyses. This research was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota, Research Grant CR807240, and by the Homestake Mining Company, Lead, South Dakota.

REFERENCES

APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, Washington, D.C.

ASTM (American Society for Testing and Materials). 1981. Proposed revision of standard methods of test for cyanide in water, Standard D-2036-81. American Society for Testing and Materials, Philadelphia, Pennsylvania.

ASTM (American Society for Testing and Materials). 1982. Designation D2036. Annual book of standards. American Society for Testing and Materials. Philadelphia, Pennsylvania.

Barnett, H.J.M., M.V. Jackson, and W.B. Spaulding. 1951. Thiocyanate psychosis. J. Am. Med. Assoc. 147: 1554-1558.

Benoit, D.A., V.R. Mattson, and D.L. Olson. 1982. A continuous-flow minidiluter system for toxicity testing. Water Res. 16: 457-464.

Boxer, G.E., and J.C. Rickards. 1952a. Determination of traces of hydrogen cyanide in respiratory air. Archs. Biochem. Biophys. 39: 287-291.

Boxer, G.E., and J.C. Rickards. 1952b. Determination of thiocyanate in body fluids. Archs. Biochem. Biophys. 39: 292-300.

Broderius, S.J., and L.L. Smith. 1980. Final Report. Grant No. R805291. U.S. Environmental Protection Agency, Duluth, Minnesota.

Davenport, H.W. 1940. The inhibition of carbonic anhydrase and of gastric secretion by thiocyanate. Am. J. Physiol. 129: 505-514.

Davies, R.E. 1951. The mechanism of hydrochloric acid production by the stomach. Biol. Rev. 26: 87-120.

Devuyst, E.A., B.R. Conard, and V.A. Ettel. 1982. Pilot plant operation of the INCO SO_2/air cyanide removal process. Can. Mining J. August 1982: 27-30.

Doudoroff, P. 1976. Toxicity to fish of cyanides and related compounds. A review. U.S. Environmental Protection Agency, Duluth, Minnesota. EPA-600/3-76-038.

Epstein, F.H., J. Maetz, and G. de Renzis. 1973. Active transport of chloride by the teleost gill: inhibition by thiocyanate. Am. J. Physiol. 224: 1295-1299.

Epstein, F.H., P. Silva, J.N. Forrest, and R.J. Solomon. 1975. Chloride transport and its inhibition by thiocyanate in gills of seawater teleosts. Comp. Biochem. Physiol. 52A: 681-683.

Garvin, C.F. 1939. The fatal toxic manifestations of the thiocyanates. J. Am. Med. Assoc. 112: 1125-1127.

Goldstein, F., and F. Reiders. 1951. Formation of cyanide in dog and man following administration of thiocyanate. Am. J. Physiol. 167: 47-51.

Goldstein, F., and F. Reiders. 1953. Conversion of thiocyanate to cyanide by an erythrocytic enzyme. Am. J. Physiol. 173: 287-290.

Goodman, L., and A. Gilman. 1970. The pharmacological basis of therapeutics. 4th ed. The Macmillan Co., London, England.

Goto, K., and D.W. Esplin. 1961. Excitatory effects of thiocyanate on spinal cord. J. Pharmacol. Exp. Ther. 133: 129-136.

Habig, W.H., J.H. Keen, and W.B. Jakoby. 1975. Glutathion S-transferase in the formation of cyanide from organic thiocyanates and as an organic nitrate reductase. Biochem. Biophys. Res. Commun. 64: 501-506.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11:714-719. Correction -- Environ. Sci. Technol. (1978) 12: 417.

Heming, T.A., R.V. Thurston, E.L. Meyn, and R.K. Zajdel. (Submitted). Acute toxicity of thiocyanate to fishes: some physiological implications. Trans. Am. Fish. Soc.

Huiatt, J.L., J.E. Kerrigan, F.A. Olson, and G.L. Potter. 1983. Workshop -- Cyanide from Mineral Processing. Utah Mining and Mineral Resources Research Institute, Salt Lake City, Utah.

Katz, A.I., and F.H. Epstein. 1971. Effect of anions on adenosine triphosphatase of kidney tissue. Enzymes 12: 499-507.

Lubin, M. 1957. The effect of iodide and thiocyanate ions on the mechanical and electrical properties of frog muscle. J. Cell Comp. Physiol. 49: 335-349.

Meyn, E.L., R.K. Zajdel, and R.V. Thurston. 1984. Acute toxicity of ferrocyanide and ferricyanide to rainbow trout (Salmo gairdneri). Technical Report No. 84-1, Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana.

Mount, D.I., and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21-29.

Pines, K.L., and M.M. Crymble. 1952. In vitro conversion of thiocyanate to cyanide in the presence of erythrocytes. Proc. Soc. Exp. Biol. Med. 81: 160-163.

Smith, R.P. 1973. Cyanate and thiocyanate: acute toxicity. Proc. Soc. Exp. Biol. Med. 142: 1041-1044.

Solomon, R.J., P. Silva, and F.H. Epstein. 1973. Thiocyanate inhibitable ATPase in the gill of Anguilla rostrata. Bull. Mt. Desert Island Biol. Lab. 13: 115-117.

Towill, L.E., J.S. Drury, B.L. Whitfield, E.B. Lewis, E.L. Galyan, and A.S. Hammons. 1978. Reviews of the environmental effects of pollutants: V. Cyanide. U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA-600/1-78-027.

U.S. EPA (U.S. Environmental Protection Agency). 1974. Methods for chemical analysis of water and wastes. U.S. Environmental Protection Agency, Washington, D.C. EPA-625/6-74-003.

U.S. EPA (U.S. Environmental Protection Agency). 1980. Ambient water quality criteria for cyanides. U.S. Environmental Protection Agency, Washington, D.C. EPA-440/5-80-037.

Vennesland, B., E.E. Conn, C.J. Knowles, J. Westley, and F. Wissing. 1981. Cyanides in biology. Academic Press, London, England.

Wolff, J. 1964. Transport of iodide and other anions in the thyroid gland. Physiol. Rev. 44: 45-90.

Zadunaisky, J.A., M.A. Lande, and J. Hafner. 1971. Further studies on chloride transport in the frog cornea. Am. J. Physiol. 221: 1832-1836.

PROCESSES OF FORMATION OF NATURAL WATER QUALITY

by

M.I. Kuz'menko and A.I. Merezhko[1]

ABSTRACT

This paper develops the notion of a unity in the processes that determine water quality in bodies of water and water courses. Relationships are examined among the productivity of a body of water, the processes of self-purification in it, and the resultant water quality. Using individual hydrobionts, in particular the higher aquatic plants and certain invertebrates, as examples, an analyis is made of their vital activities in relation to the processes that determine natural water quality. Significant attention is given to a description of gas conditions in the body of water in relation to photosynthesis in the higher aquatic plants. The role of individual hydrobionts in the absorption and accumulation of biogenic elements and toxic compounds is shown. Particular attention was directed to the role of the root systems of plants and to the filtration capacity of dreissensia (zebra mussel) in these processes. The conclusion is drawn that water quality is a product of the functioning of the ecosystem as a whole.

INTRODUCTION AND DISCUSSION

A water reservoir is a complicated ecological system with more or less marked and, not infrequently, quite discrete links between its elements. The linkages are determined by the nature of hydrological, physicochemical and biological processes that provide for the function of the ecosystem as an integral whole.

The productivity of the body of water and the quality of water in it are integral indicators of function of aquatic ecosystems. While reservoir productivity is closely related to its trophism, water quality is related to its self-purification processes. The latter takes place as a result of biotic circulation of matter (Vinberg 1976), which includes formation,

---

[1] Institute of Hydrobiology, Ukrainian Academy of Sciences,

conversion, and destruction of organic compounds. The quantitative expression of these processes and the relationship between them determines the direction of self-purification.

Formation of an organic substance in water (photosynthesis) is related to carbon dioxide uptake, use of solar energy, and absorption of biogenic elements. The relationship between these processes indicates that the more intensive plant photosynthesis is, the more active is the absorption of biogenic elements.

An excess or shortage of these elements leads to disturbances of intrareservoir processes related to self-purification and formation of the quality of naturally occurring water.

As a result of photosynthesis by aquatic plants alone, more than 400 billion tons of oxygen is discharged per year, and it would be difficult to exaggerate its role in formation of water quality. According to our data, perfoliate pondweed gives off about 50 mg oxygen/h/100 g wet weight during photosynthesis. The discharged oxygen is instrumental in reaeration of water (Vinberg 1976) and provides rheophil conditions for hydrobionts.

Not infrequently, one observes oversaturation of water with oxygen in macrophyte thickets, particularly around noon. Such conditions provide for normal vital function of hydrobionts and supply oxygen for processes of oxidation of organic substances, especially in the summer. With elevation of temperature in thickets of macrophytes, there is activation of microbiological processes. There is intensification of vital functions of periphyton, which also improves oxygen conditions. Even if there are instances of oxygen deficiency in macrophyte thickets, they are local and temporary. According to our data, the oxygen shortage disappears with the first rays of the sun, and by noon to 1 pm one observes oversaturation of water with oxygen in thickets of macrophytes. The prevalence of processes of oxygen uptake by higher aquatic plants over its production is observed for only 5-6 h (Figure 1).

Analysis of the curves of output and uptake of oxygen by higher aquatic plants warrants the conclusion that the amount of oxygen discharged by submerged macrophyte species is more than 16 times greater than the amount taken up. This can be expressed as ${}^{P}O_2/{}^{R}O_2 = 3.1$ for perfoliate pondweed and ${}^{P}O_2/{}^{R}O_2 = 16$ for hornwort. Hence, it can be concluded that oxygen given off during photosynthesis in higher aquatic plants is used to a significant extent, not only for plant respiration, but for formation of oxygen conditions in the water.

One also observes a correlation between photosynthesis of higher aquatic plants and formation of chemical composition of water. This link is manifested by a change in carbonate equilibrium and assimilation of different forms of carbon dioxide by assimilating plants. Bicarbonates are almost always present in natural waters. When there is intensive uptake by plants, free carbon dioxide disappears very rapidly from water, and the plants change to assimilation of bicarbonate carbon dioxide, which again leads to formation of carbonates. This is associated with change in either direction

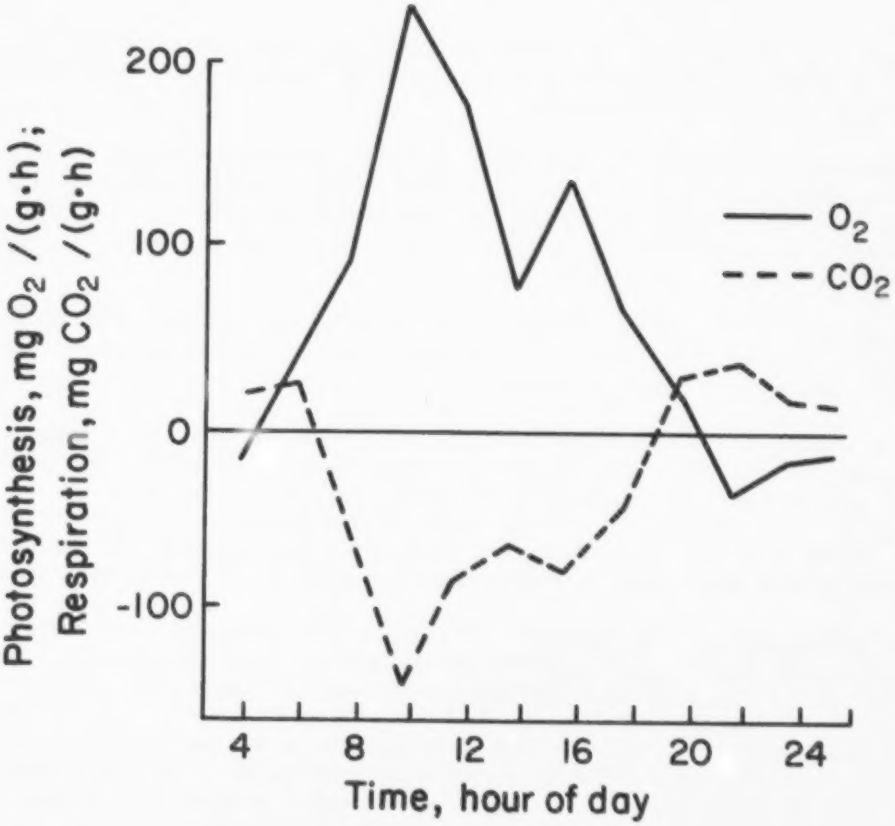


FIGURE 1. Daily exchange of gases in the hornwort.

in concentration of hydrogen ions (Merezhko 1967, Shiuyan and Merezhko 1972), which in turn, affects the direction of processes of formation of water quality. But the biological essence of photosynthesis in processes of formation of quality of natural water is not limited to this.

One of the distinctions of all photosynthetic organisms is their capacity to absorb and accumulate in tissues and organs a significant quantity of various biogenic elements. The role of this process in the cycle of biogenic elements and in forming water quality is unquestionable. The figure characterizing accumulation of biogens by aquatic plants fluctuates over a rather wide range, and it depends on a number of biotic and abiotic factors. For this reason, the participation of higher aquatic plants in processes of formation of water quality is permanent.

The direction of intrawater processes determining formation of water quality depends largely on the period of turnover of biogenic elements. The latter, in turn, is determined by the physiological activity of biogens, intensity of their absorption, and duration of the period of accumulation in plants. Nitrogen, phosphorus, potassium, iron, chlorine, and manganese are utilized the most actively by plants. The same elements are deciding factors in processes of formation of water quality (Merezhko 1973).

Nitrogen and phosphorus accumulate in virtually all plants in the same amounts (Table 1). All other biogens are absorbed and accumulated by different species of higher aquatic plants in different amounts. There is particularly distinct demonstration of differences in uptake and accumulation of potassium, magnesium, calcium, manganese, and iron. They are taken up the most intensively by the lesser reedmace, bulrush, flowering rush, common water plantain and perfoliate pondweed, and to a lesser extent by the common reed.

We should dwell in particular on chlorine uptake by plants. Virtually all of the tested plant species absorb and accumulate it in a rather large quantity. The amount of chlorine in plants ranges from 1.0 to 1.8%. It is absorbed the most by the common water plantain.

Absorbed biogens are unevenly distributed in the plant (Table 2). Thus, in August, maximum levels of nitrogen, iron, and manganese were demonstrated in leaves and reproductive organs of the reed. There are lower levels of these biogens in its stems and rhizomes. The opposite is observed at the end of the vegetation period. At this time, there is efflux of biogens from leaves and stems into the root system. The latter is extremely important to processes of formation of water quality, since a surplus of biogenous elements in a reservoir is bound, to some extent, with plant root systems and is thus excluded from circulation for a relatively long time.

Other autotrophic organisms, including algae, play an analogous role in processes of circulation of biogens in aquatic ecosystems. The generation period is very short, however, in algae and other autotrophic organisms.

After the plants die off, absorbed minerals and organic matter return into water and become actively involved in the cycle of matter. The role of this group of autotrophs in formation of water quality, like that of higher aquatic plants, is also manifested by their participation in processes of formation of biologically valuable water. Probably, algae play the first and foremost role in this process. Their role in discharging biologically active compounds, including enzymes, into the environment and in the formation of water quality is well-known and proven (Telitchenko 1972, Telitchenko and Telitchenko 1972). Biologically valuable water can be formed only under the influence of hydrobionts.

Concurrently with uptake of biogenous elements, autotrophs take up, accumulate, and transform toxic compounds. The most importance in this process is given to higher aquatic plants (Merezhko et al. 1975). There is both passive and active absorption of these compounds by aquatic macrophytes. Passive absorption occurs during transpiration, when one cannot demonstrate transformation of these compounds in plants. Consequently,

TABLE 1. ACCUMULATION OF SOME BIOGENOUS ELEMENTS IN HIGHER AQUATIC PLANTS, % DRY MATTER

Object of study		P	K	Ca	Mg	Na	Cl	Si	Fe, mg%	Mn, mg%
Common reed	2.17 ±0.04	0.35 ±0.02	1.70 ±0.03	0.38 ±0.002	0.10 ±0.01	0.14 ±0.02	1.36 ±0.01	1.13 ±0.02	0.005 ±0.0009	0.02 ±0.001
Lesser reedmace	2.52 ±0.06	0.41 ±0.01	1.19 ±0.01	1.07 ±0.01	0.15 ±0.01	0.51 ±0.02	1.20 ±0.02	0.12 ±0.01	0.01 ±0.001	0.06 ±0.001
Bulrush	2.34 ±0.06	0.39 ±0.01	2.35 ±0.07	0.89 ±0.02	0.12 ±0.01	0.40 ±0.03	1.56 ±0.04	0.31 ±0.01	0.006 ±0.0007	0.03 ±0.0001
Flowering rush	2.66 ±0.07	0.40 ±0.01	4.36 ±0.09	1.36 ±0.06	0.21 ±0.02	0.43 ±0.01	1.17 ±0.02	0.34 ±0.03	0.03 ±0.002	0.09 ±0.002
Common water plantain	2.09 ±0.08	0.55 ±0.02	2.89 ±0.08	1.20 ±0.4	0.16 ±0.01	0.36 ±0.02	1.87 ±0.03	0.73 ±0.04	0.01 ±0.0001	0.06 ±0.0001
Perfoliate pondweed	2.02 ±0.02	0.53 ±0.03	2.01 ±0.05	0.95 ±0.02	0.33 ±0.02	0.33 ±0.01	1.55 ±0.02	0.42 ±0.03	0.01 ±0.001	0.03 ±0.0001

TABLE 2. CHEMICAL COMPOSITION OF COMMON REED ORGANS (AUGUST)

	Plant Organs			
Composition	Leaves	Stems	Panicles	Rhizomes
Ash	6.91	3.89	7.96	6.17
Compound, % dry matter				
N	2.48	0.85	1.45	1.46
P	0.26	0.13	0.39	0.19
K	0.70	0.48	0.99	2.28
Ca	0.98	0.26	0.35	0.15
Mg	0.11	0.05	0.09	0.03
Na	0.06	0.05	0.03	0.15
Cl	0.57	0.74	0.73	1.17
Si	0.24	0.17	2.32	6.17
Compound, mg% dry matter				
Fe	14.04	11.58	21.75	8.07
Mn	46.84	11.68	25.58	6.20

passive absorption of toxic compounds by macrophytes provides, to some extent, only for migration of toxic agents from one environment to another --in this case, from water to air. However, active absorption of toxic compounds by macrophytes leads to their complete or partial detoxification. This already has a direct bearing on processes of self-purification and formation of water quality.

Carbon-labeled DDT and sevin are demonstrable in plants as early as 1 day after their introduction into the environment. The highest concentrations of these agents have been found in the root system of plants and somewhat lower ones in leaves and stems (Table 3).

The data on presence of the radioactive tracer in reed organs warrant the assumption that DDT and sevin probably enter the plant through its root system. The arrangement of the reed's root system is somewhat differ-

TABLE 3. UPTAKE AND ACCUMULATION OF ^{14}C-LABELED DDT AND SEVIN BY COMMON REED (Activity of specimens in thousands of pulses/min·g)

Pesticide	Plants with water roots			Plants without water roots	
	Leaves	Stems	Water roots	Leaves	Stems
DDT	2.4 ± 0.05	5.6 ± 0.9	540.2 ± 11.0	7.3 ± 0.5	8.4 ± 0.3
Sevin	2.1 ± 0.9	2.8 ± 0.7	29.7 ± 2.5	1.7 ± 0.3	0.9 ± 0.2

ent from that of other plants. Part of its roots lie along the bottom of the body of water (water roots). These roots are markedly ramified, and they absorb the most actively both biogenous elements and toxic compounds (Table 4, Figure 2). Toxic compounds are accumulated just as actively by reed rhizomes, and this is important to processes of formation of water quality. It can be assumed that, because of this, higher aquatic plants can serve under certain conditions for detoxification of some deleterious substances in the water environment (Merezhko 1973).

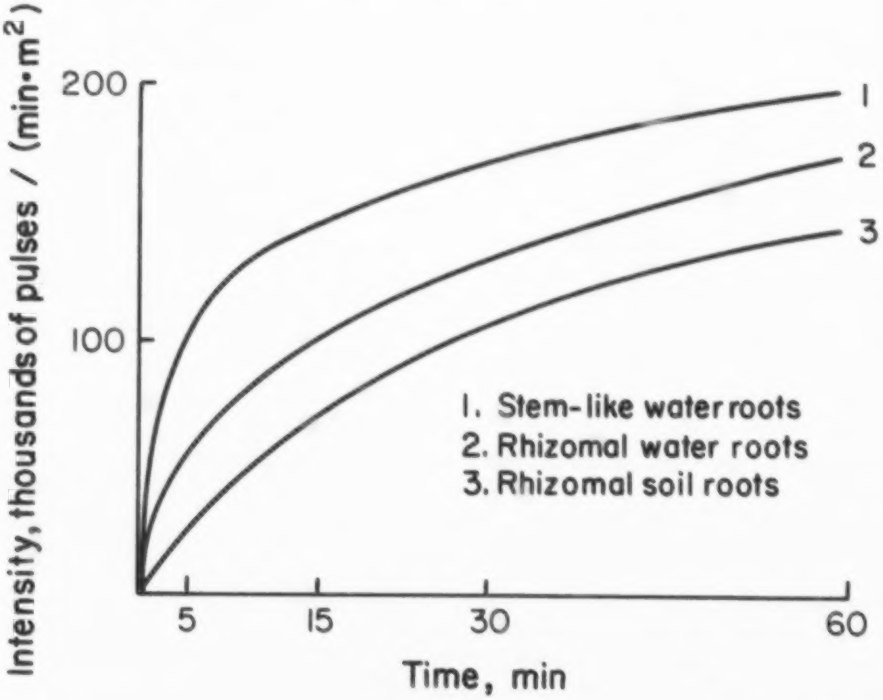


FIGURE 2. Intensity of uptake of ^{14}C of alanine-I-^{14}C by adventitious roots of common reed.

TABLE 4. DISTRIBUTION OF ^{14}C IN COMMON REED ROOT SYSTEM, THOUS. PULSES/MIN·G DRY WEIGHT

Pesticide	Pesticide Concentration mg/ℓ	Rhizomes	Radioactivity of leaves, %	Water roots	Radioactivity of leaves, %	Soil roots	Radioactivity of leaves, %
DDT	0.50	0.83 ± 0.03	6.7	0.45 ± 0.00	3.6	0.08 ± 0.01	0.69
	1.00	0.81 ± 0.02	6.8	0.53 ± 0.0	4.4	0.12 ± 0.0	1.0
	2.00	1.01 ± 0.04	10.6	0.93 ± 0.01	9.8	0.18 ± 0.0	1.9
HCCH*	0.50	1.20 ± 0.03	16.9	1.02 ± 0.01	14.3	0.09 ± 0.0	1.2
	0.25	1.11 ± 0.06	15.0	0.86 ± 0.0	11.6	0.06 ± 0.0	0.8
	1.00	1.53 ± 0.07	24.3	1.45 ± 0.05	23.0	0.12 ± 0.0	1.9
Control	--	0.98 ± 0.01	5.9	0.37 ± 0.0	2.2	0.70 ± 0.0	0.4

*Hexachlorocyclohexane

While we wish to stress the role of higher aquatic plants, as well as other autotrophic organisms, in processes of formation of surface water quality, it must be borne in mind that this role can be positive only if plants are promptly harvested from reservoirs (Frantsev 1969, 1972). Thus, according to some simple estimates, more than 200 kg nitrogen, 80 kg phosphorus, over 130 kg chlorine, 60 kg calcium, etc., are removed per hectare when harvesting reed vegetation from water.

In describing processes of formation of quality of natural waters as related to vital functions of hydrobionts, special attention must be given to microorganisms and invertebrates with respect to uptake and accumulation of biogenous elements and organic compounds (Kryuchkova 1972, Vinberg 1972). Mollusks play a special role in these processes as filters and sedimentators of suspended substances.

The results of many years of investigation of Dreissena polymorpha (Shevtsova and Kharchenko 1981) indicate that this mollusk is a powerful filter for suspended particles. On this basis, some authors believe that the Dreissena plays an important part in biological self-purification (Stanezykowska 1975, Kondrat'yev 1977, Shevtsova and Kharchenko 1981). We cannot help but agree with this, but one should not forget that, in the absence of a consumer of Dreissena, its role in both self-purification processes and formation of water quality could be virtually eliminated and, in some cases (hyperproduction) it could be negative. Nevertheless, the significance of this mollusk in processes of formation of water quality in regions where it develops on a mass scale is quite obvious. According to the data of Shevtsova and Kharchenko (1981), 1 m^2 of Dreissena on the bottom of a canal filters 405.9 m^3 of water per year. This is associated with up to 0.747 kg mineralization and precipitation of 7.7 kg organic substances in the form of agglutinates. The role of invertebrates in processes of formation of water quality, like that of plants, is determined by the direction and intensity of their metabolic processes. The role of higher aquatic plants has also been proven in processes of precipitation of suspended matter (Figure 3) (Merezhko 1978).

Equal importance to formation of water quality is attributed to processes of destruction and mineralization of organic matter. It should be borne in mind, however, that not all organic substances of autochthonous and allochthonous origin undergo mineralization. A significant part of them accumulates in the form of inoxidizable fractions, which often have a direct bearing on processes of formation of water quality (Telitchenko 1972).

Microflora and chemical oxidation play the leading role in destruction and mineralization of organic matter. The relative involvement of different groups of microorganisms in destruction and mineralization of organic matter is not the same, and it is determined by the type of reservoir and amount of substance being mineralized (Vinberg 1972).

Thus, the structural and functional organization of circulation of matter in aquatic ecosystems is affected by hydrobionts on different trophic levels by the principle of waste-free technology.

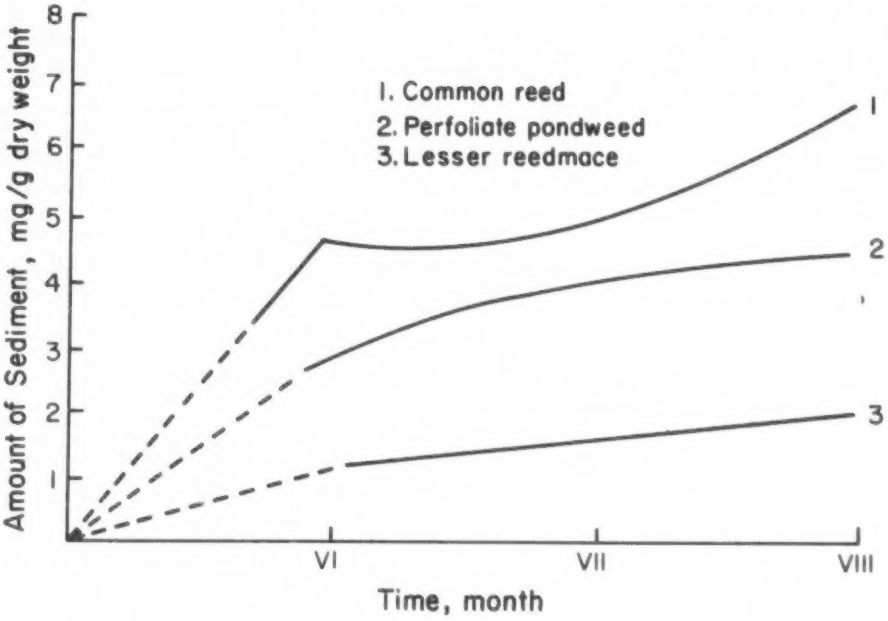


FIGURE 3. Precipitation of suspended matter on higher aquatic plants during vegetation period.

In the case of intensive operation of water resources for recovery of the basic product of an aquatic ecosystem--biologically valuable water, it is necessary to control quantitative and qualitative development of biohydrocenoses. Consideration of specifics of function and control of development of dominant representatives of producers and consumers as a function of time and space provides a possibility for selecting optimum methods of formation of water quality and improving the efficiency of operating marine ecosystems.

BIBLIOGRAPHY

Frantsev, A.V. 1969. One must help nature! Khimiya I Zhizn'. No. 4.

Frantsev, A.V. 1972. Some problems of controlling water quality. In: Teoriya i praktika biologicheskogo samoochishcheniya zagryaznennykh vod. Moscow, USSR.

Frobisher, M. 1965. Osnovy mikrobiologii (Bases of Microbiology), Moscow, USSR.

Kondrat'yev, G.P. 1977. Biofiltration. In: Volgogradskoye vodokhranilishche: naseleniye, biologicheskoye prognozirovaniye i samoochishcheniye (The Volgograd Reservoir. Population, Biological Forecasting and Self-Purification). Saratov, USSR.

Kryuchkova, N.M. 1972. Zooplankton as an agent in self-purification of water reservoirs. In: eoriya i praktika biologicheskogo samoochishcheniya prirodnykh vod (Theory and Practice of Biological Self-Purification of Naturally Occurring Waters). Moscow, USSR.

Merezhko, A.I. 1967. Ecological and physiological distinctions of blue-green algae. Abstract of candidatorial dissertation--biological sciences. Kiev, USSR.

Merezhko, A.I. 1973. Role of higher aquatic plants in formation of water quality. Gidrobiol. Zhurn. 9(4).

Merezhko, A.I. 1976. Ecological and physiological distinctions of higher aquatic plants and their role in formation of water quality. Abstract of doctoral dissertation--biological sciences. Kiev, USSR.

Shevtsova, L.V. and T.A. Kharchenko, T.A. 1981. Role of Dreissena in processing suspended organic matter in the North Crimea Canal. Gidrobiol. Zhurn., 17(5).

Shiyan, P.N. and A.I. Merezhko. 1972. Effect of concentration of hydrogen ions on photosynthesis and metabolism of radioactive carbon in aquatic plants. Gidrobiol. Zhurn. 8(2). Ibid, Vol. 8, No. 2, 1972.

Stanezykowska, A. 1975. Ecosystem of the Mikolajekie Lake. Regularities of the Dreissena Polymorpha Poll. (Bivalvia) Occurrence and its function in the lake. Pol. Arch. Hydrobiol. No. 22.

Telitchenko, M.M. 1972. Possibility of controlling self-purification processes using biological methods. In: Teoriya i praktika biologicheskogo samoochishcheniya zagryaznennykh vod. Moscow, USSR.

Telitchenko, M.M. and L.A. Telitchenko. 1972. The problems of water quality and current methods of solving them. In: Teoriya i praktika biologicheskogo samoochischen iya zagryaznennykh vod. Moscow, USSR.

Vinberg, G.G. 1972. Significance of hydrobiology to solution of water management problems. In: Teoriya i praktika biologicheskogo samoochishcheniya zagryaznennykh vod (Theory and Practice of Biological Self-Purification of Polluted Water). Moscow, USSR.

Vinberg, G.G. 1976. Biologicheskiye protsessy samoochishcheniya na zagryaznennom uchastke reki (Biological Self-Purification Processes in Polluted River Section). Minsk, USSR.

CO_2 EXCRETION AND AMMONIA TOXICITY IN FISHES: IS THERE A RELATIONSHIP?

by

T.A. Heming[1]

ABSTRACT

The aquatic toxicity of ammonia is pH-dependent and, therefore, may be modulated by chemical-biological reactions, such as hydration of excreted CO_2, which alter the pH of water adjacent to the gill surface. Effects of CO_2 excretion on the pH of interlamellar water of rainbow trout (Salmo gairdneri) were investigated by following changes in the pH of mixed expired water (pHex) using a stopped-flow apparatus. The pHex immediately postgill was approximately the same as inspired water pH, but pHex decreased as a nonlinear function of time downstream of the gill. Half-time of the acidification reaction was 240 sec, in excellent agreement with the half-time of uncatalyzed $CO_2:HCO_3^-$ equilibrium. Acetazolamide (0.44 mM) in the bathing medium had no effect on slow downstream changes in pHex, indicating that branchial carbonic anhydrase was not available to catalyse hydration of excreted CO_2. Downstream changes in pHex were abolished by external carbonic anhydrase (28,080 Wilbur-Anderson units/L). These results suggest that CO_2 excretion has little, if any, acidifying effect on water adjacent to the gill surface. Measurements of bulk water pH are probably the best possible indicators of boundary layer pH. Attempts to explain the effect of pH on the LC50 concentration for ammonia in terms of a significant difference between the pH of bulk water and gill water are over-simplified to the point of being erroneous.

INTRODUCTION

The relative toxicity of ammonia solutions ($NH_4^+ + H_2O = NH_3 + H_3O^+$, $H_3O^+ = H^+$) to fishes increases when environmental pH increases (Lloyd and

---

[1] Department of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 2A9. Present address: Aquatic Toxicology Section, Alberta Environmental Centre, Vegreville, AB, Canada T0B 4L0.

Herbert 1960, U.S. EPA 1977). This pH-dependency is related to an increase in the ratio of non-ionized to ionized ammonia (NH_3:NH_4^+) at higher pH values (Emerson et al. 1975). Biological membranes, such as the fish gill, are generally more permeable to non-ionized compounds than to ionized compounds (Jacobs 1940). If one assumes, on the basis of this difference in gill permeability, that NH_3 is the sole toxic species in ammonia solutions then it follows that the LC50 concentration for such solutions in terms of the concentration of NH_3 should be independent of pH. Various studies (Lloyd and Herbert 1960, Tabata 1962, Tomasso et al. 1980, Thurston et al. 1981), however, have shown that the LC50 concentration for NH_3 is positively correlated with environmental pH, which suggests that NH_3 is more toxic to fish at lower pH values. There are several possible explanations for this trend. Firstly, the assumption that NH_3 is the sole toxic species in ammonia solutions may be incorrect. NH_4^+ may also exert a toxic effect that would become more apparent at lower pH values as the relative proportion of NH_4^+ in solution increases. Secondly, past calculations and measurements of the concentration of NH_3 in bulk water may be inappropriate estimates of the concentration of NH_3 at the gill surface.

The ratio of NH_3:NH_4^+ in solution is dependent upon the solution pH, temperature and ionic strength (Emerson et al. 1975). Because of heat and mass transfer across the gill, the chemical characteristics of boundary layer water at the gill surface must differ from that of bulk water for thermodynamic reasons (Buysman and Koide 1971). Such differences in water temperature and ionic strength probably are extremely minute and have negligible effects on NH_3:NH_4^+ equilibrium. More importantly, several authors (Lloyd and Herbert 1960, Szumski et al. 1982) have suggested that water adjacent to the gill surface is significantly more acidic than bulk water due to excretion of CO_2 and a resultant CO_2 gradient normal to the gill surface. The proposed differences are as large as 0.31 pH units (Lloyd and Herbert 1960).

For hydration of metabolic CO_2 ($CO_2 + H_2O = H^+ + HCO_3^-$) to have such an appreciable effect on boundary layer pH, the hydration reaction must occur rapidly enough to alter the proton concentration within the interlamellar transit time of gill water (0.10 - 10.15 sec, Randall et al. 1982). This is much faster than the uncatalysed rate of CO_2 hydration (Kern 1960). Szumski et al. (1982), however, have proposed that this CO_2 reaction is catalysed by gill carbonic anhydrase. These authors have developed a model to estimate the concentration of NH_3 at the gill surface taking into account biological CO_2 excretion and local water quality. Depending upon water quality characteristics, the model of Szumski et al. (1982, see their Figure 6) predicts that the U.S. Environmental Protection Agency's criterion for total ammonia (U.S. EPA 1977) for protection of warm water fishes is 6 to 39 times too restrictive. CO_2-induced acidification of water adjacent to the gill, however, does not fully account for observed effects of pH on the toxicity of NH_3 (Roseboom 1983) or other acids/base (Broderius et al. 1977). Moreover, there is nothing in the experimental literature that allows one to quantify the effects of CO_2 excretion on the pH of interlamellar water.

The aim of the present study was to investigate the effects of CO_2 excretion on the pH of water adjacent to the gill of rainbow trout (Salmo gairdneri) by following changes in the pH of mixed expired water. If CO_2 hydration at the gill surface occurs rapidly enough to have an appreciable effect on interlamellar water pH, the pH of mixed expired water (pHex) will be lower than inspired water pH (pHin) and no downstream changes in pHex will occur. On the other hand, if CO_2 hydration at the gill surface occurs slowly, pHex immediately postgill will be approximately equal to pHin and pHex will decrease slowly downstream of the gill due to continued CO_2 hydration. Downstream changes in pHex were followed using a stopped-flow apparatus. Effects of external carbonic anhydrase and acetazolamide, a specific inhibitor of carbonic anhydrase, on downstream changes in pHex were also examined.

METHODS

Rainbow trout (Salmo gairdneri) weighing between 200 and 400 g were obtained from Sun Valley Hatchery (Mission, B.C.). The fish were housed outdoors in tanks supplied with continuous flows of dechlorinated Vancouver tapwater (temperature 6 - 10°C) and were fed to satiation daily with a commercial trout food. Fish were starved for at least 48 hours prior to and during the experiments.

Fish were anaesthetized (50 - 67 mg/L tricaine methanesulfonate, pH adjusted to 7.5 with $NaHCO_3$, temperature 8°C) and fitted with catheters for sampling of mixed expired water. Catheters were constructed from polyethylene tubes (inner diameter 0.86 mm, length 15 - 20 cm) that had been heat-sealed at one end. Numerous small holes were made along a 3 to 4 cm length of the tube near the sealed end. Catheters were sutured to the fish, one catheter per fish, in such a way that these holes were located immediately behind the left opercular cover and spanned the entire length of the opercular cleft, each hole facing anteriorly into the opercular cavity. Following surgery, each fish was transferred to a 2-L tank supplied with a 1.5 to 2.0 L/min flow of test water that was recirculated from a thermostated (8 ± 0.5°C) 120-L reservoir. Test water was renewed at a rate of approximately 25% every 2 days. The test water was a balanced salt solution consisting of 40 mM NaCl, 1.6 mM KCl, 0.47 mM $CaCl_2$, 0.62 mM $MgSO_4$, 5.5 mM $NaHCO_3$ and 0.95 mM NaH_2PO_4 in dechlorinated Vancouver tapwater. The buffering value of this test water was about 0.508 mM H^+/pH unit in the pH range 7.2 to 8.5. Experiments were conducted in this test water, rather than in normal freshwater, because the presence of dissolved salts greatly improved the stability, reproducibility and response time of water pH measurements. Fish were allowed 7 to 10 days to acclimate to the test water before experiments were begun. This period of time is more than adequate to allow rainbow trout to re-establish ionic, osmotic and respiratory steady-state conditions after transfer from freshwater to balanced salt solutions (Perry and Heming 1981).

Water pH was measured using a polymer body, sealed reference combination pH microelectrode (Canlab) and a Radiometer PHM64 meter, and was simultaneously recorded using a chart recorder. The pH electrode was housed in an acrylic plastic measurement chamber (measurement volume 0.1 mL) that was situated in the test water upstream of the fish (Figure 1). The inlet port of this chamber communicated directly with the test water when measurements

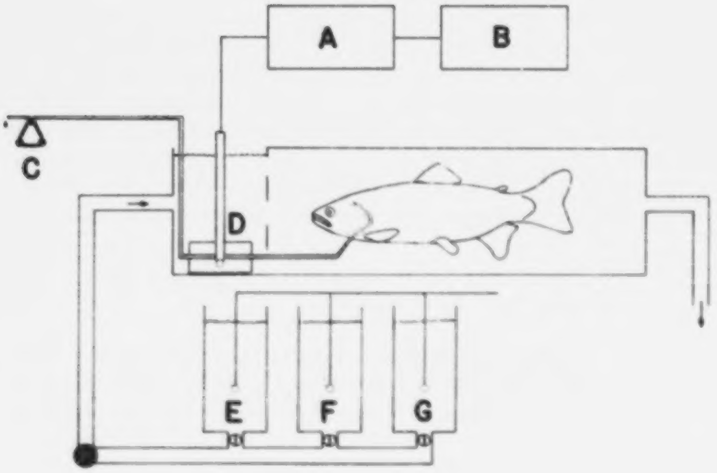


Figure 1. Outline of experimental apparatus used to measure the pH of mixed expired water of trout. A. pH meter; B. chart recorder; C. peristaltic withdrawal pump; D. reference combination pH electrode; E, F, and G, reservoirs of test water with and without 28,080 Wilbur-Anderson units/L bovine carbonic anhydrase or 0.44 mM acetazolamide.

of inspired water pH (pHin) were made and was connected to the opercular catheter when measurements of mixed expired water pH (pHex) were made. In both situations, the outlet port of the chamber was connected to a peristaltic pump that withdrew water past the electrode at a rate of 5 mL/min. At that pumping rate, water samples required less than 1.8 sec to transit the opercular catheter and about 1.2 sec to fill the measurement chamber. Response time of the pH-measuring system was determined by rapidly switching the inflow to the measurement chamber between 20 mM phosphate buffers with pH values of 4.05 and 7.00; half-time of the resultant response was 12.5 sec. This response time was 10 to 20 times faster than the expected half-time of the uncatalysed $CO_2:HCO_3^-$ reaction and so was deemed adequate for my purposes.

A typical experiment consisted of the following measurements. Inspired water pH was measured under continuous-flow conditions for 20 to 30 minutes. The withdrawal pump was then switched off, suddenly stopping water flow past the electrode, and pHin was followed under stopped-flow conditions. The electrode assembly was then connected to the opercular catheter and pHex was measured under identical continuous-flow and stopped-flow conditions. Water flow through the fish tank was subsequently switched to a thermostatted (8 ± 0.5°C) 4-L reservoir of test water that contained 28,080 Wilbur-Anderson units/L; bovine carbonic anhydrase, and pHin and pHex were again measured under continuous-flow and stopped-flow conditions. Water flow through the fish tank was then returned to the control reservoir for a period of 1 to 2 hours. Finally, water flow through the fish tank was switched to a thermostatted

(6 ± 0.5°C) 4-L reservoir of test water that contained 0.44 mM acetazolamide, and pHin and pHex were again measured under continuous-flow and stopped-flow conditions.

Observed changes in water pH were quantified by two methods. Firstly, the value for dpH/dtime was calculated at the point when water flow past the electrode was first stopped. The initial rate of H^+ generation was calculated as the product of that value and the buffering value of the test water. Secondly, the quantity of H^+ generated at 1-minute intervals after electrode flow was stopped was calculated as $vB(pHex^t - pHex^o)$, where $pHex^o$ was the pHex under continuous-flow conditions, $pHex^t$ was the pHex at known time intervals after electrode flow was stopped, v was the measurement volume, and B was the buffering value of the test water. These data were fitted by the methods of least squares to a double-reciprocal plot, which allowed calculation of the half-time of the H^+ generating reaction.

The data are presented as arithmetic means ± their standard errors (SE). Means were compared statistically using paired Students t-tests. A 5% level of probability ($P \leq 0.05$) was adopted as the fiducial level of significance.

RESULTS

The pH of inspired water was unaffected by water flow past the pH electrode, pHin measurements made under continuous-flow and stopped-flow conditions were stable with time and did not differ from one another. The pH of mixed expired water, on the other hand, was affected by flow conditions (continuous versus stopped) in control and acetazolamide studies (Figure 2). In all control and acetazolamide studies, pHex decreased as a nonlinear function of time once water flow past the electrode was stopped. Flow conditions had no effect on pHex in carbonic anhydrase studies.

Kinetics of the changes in pHex under stopped-flow conditions are summarized in Table 1. No statistical differences were present between the pHex changes in control and acetazolamide studies. In these studies, $pHex^o$ (mixed expired water pH under continuous-flow conditions) did not differ significantly from pHin. The pHex became progressively more acidic than pHin, however, once electrode flow was stopped in control and acetazolamide studies. The initial rate of acidification in these studies averaged 0.943 nmol H^+/min, the half-time of the acidification reaction averaged 240 sec, and the final equilibrium pHex averaged 0.12 units lower than pHin. Similar changes in pHex were not seen in carbonic anhydrase studies. In carbonic anhydrase studies, $pHex^o$ was consistently lower than pHin by about 0.12 units, and pHex remained stable at that value under stopped-flow conditions.

DISCUSSION

Mixed expired water of rainbow trout was not in pH equilibrium when it exited the opercular cavity. The pH of mixed expired water immediately upon exiting the opercular cavity was approximately that of inspired water. Mixed expired water became progressively more acidic than inspired water downstream of the opercular cavity at a relatively slow rate. The half-time

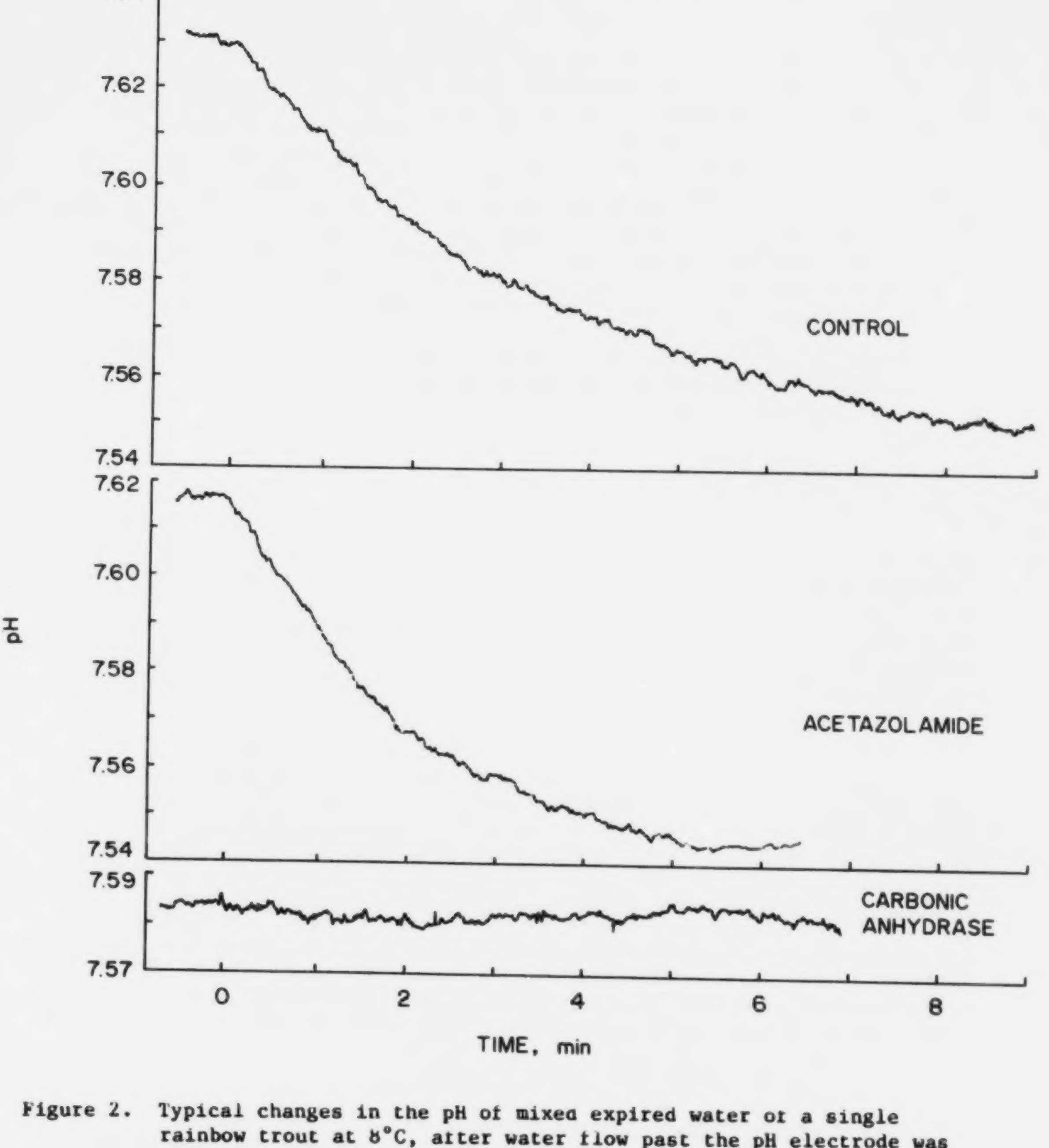


Figure 2. Typical changes in the pH of mixed expired water of a single rainbow trout at 8°C, after water flow past the pH electrode was stopped at time 0. The trout was exposed sequentially to control test water, test water containing 28,080 Wilbur-Anderson units/L bovine carbonic anhydrase, (90 minutes rinse in control test water, and test water containing 0.44 mM acetazolamide.

TABLE 1. KINETICS OF CHANGES IN THE pH OF MIXED EXPIRED WATER OF RAINBOW TROUT AT 8°C, MEASURED UNDER STOPPED-FLOW CONDITIONS. VALUES ARE MEANS ± SE (N = 4). pHin, INSPIRED WATER pH; pHex°, EXPIRED WATER pH UNDER CONTINUOUS-FLOW CONDITIONS; NS, NO SIGNIFICANT TRENDS IN pH WERE OBSERVED.

pHin	pHex	Final equilibrium pH	Initial rate of H^+ generation (nmol/min)	Half-time of H^+ generation (sec)
		Control		
7.64 ± 0.03	7.63 ± 0.06	7.52 ± 0.07	0.89 ± 0.04	270 ± 32
		Acetazolamide		
7.67 ± 0.04	7.66 ± 0.01	7.56 ± 0.02	1.00 ± 0.07	210 ± 13
		Carbonic anyhdrase		
7.69 ± 0.04	7.57 ± 0.03	7.58 ± 0.04	0.05 ± 0.02	NS

of the acidification reaction was approximately 240 sec at 8°C and an average pHin of 7.66. This value is in excellent agreement with the half-time for the uncatalysed equilibrium of CO_2 and HCO_3^- (Table 2). Downstream acidification of mixed expired water indicates that the CO_2:HCO_3 equilibrium was dominated by hydration of excreted CO_2. This is consistent with the model of Cameron and Polhemus (1974) for CO_2 excretion in fish, which predicts that molecular CO_2 accounts for 95% of total carbon dioxide excretion while HCO_3^- efflux accounts for only the remaining 5%. Assuming the observed acidification reaction obeyed first-order kinetics and given the one-for-one stoichiometry between CO_2 and H^+ in the CO_2 hydration reaction, one calculates from the initial rate of H^+ generation, the reaction half-time and the CO_2 solubility coefficient for water (Boutilier et al. 1984) that the change in Pco_2 (partial pressure of CO_2) between inspired and mixed expired water of

TABLE 2. HALF-TIME (SEC) FOR UNCATALYSED CO_2:HCO_3^- EQUILIBRIUM IN AQUEOUS SOLUTIONS AT INFINITE DILUTION, DETERMINED BY LINEAR INTERPOLATION OF DATA OF KERN (J. Chem. Educ., 37:14-23, 1960) AT 0 and 25°C.

	Temperature (°C)		
pH	0	8	25
4.00	1.3	0.94	0.18
5.00	10	7.2	1.3
6.00	74	53	9.1
7.00	240	172	26
7.64	--	198	--
8.00	300	212	25
9.00	200	139	8.7
10.00	47	32	1.1
11.00	5.4	3.7	0.12

rainbow trout at 8°C was about 0.7 torr*. This is a reasonable value considering the arterial-venous difference in blood Pco_2 across the trout gill (0.5-1.0 torr, Heming 1984), CO_2 production by gill tissue and the diluting effect

---

*According to first-order kinetics, reaction velocity (R, mol/L/sec) is related to substrate concentration (c, mol/L) as

$$R = kc,$$

where k is the rate constant. The half-time (t, sec) of such a reaction is calculated as

$$t = \ln 2 / k.$$

Given values for R and t, these equations can be rearranged to calculate the initial substrate concentration as

$$c = Rt / \ln 2.$$

The concentration of CO_2 determined in this way was converted to its partial pressure by division with the appropriate solubility coefficient. Since CO_2 and HCO_3^- in inspired water were in complete equilibrium and, therefore, did not contribute to the observed changes in pHex, the above calculations do not require that the Pco_2 of inspired water be known. Rather, these calculations yield the increase in Pco_2 as water passes over the gills.

of inspired water that bypasses the secondary gill lamellae. This suggests that the majority of excreted CO_2 was hydrated at an uncatalysed rate downstream of the gill; one would have expected an unreasonably low estimate of the difference in inspired:expired water Pco_2 if excreted CO_2 was hydrated in a partially catalysed equilibrium at the gill surface. The finding that external acetazolamide had no effect on downstream changes in pHex indicates that branchial carbonic anhydrase was not available to catalyse CO_2:HCO_3 interconversions within the boundary layer at the gill surface. This is consistent with the results of histochemical surveys of carbonic anhydrase in branchial and opercular epithelia of fish (Haswell et al. 1980, Dimberg et al. 1981, Lacy 1983). These studies have shown that the enzyme is localized in the cytoplasm and nuclei of epithelial cells; no evidence of carbonic anhydrase on the apical surface of branchial or opercular epithelia has been found. Results of the present carbonic anhydrase studies provide further evidence that slow changes observed in pHex were due to continued hydration of CO_2 downstream of the opercular cavity. Mixed expired water attained pH equilibrium prior to exiting the opercular cavity only when external carbonic anhydrase was available to catalyse CO_2:HCO_3 interconversions within the interlamellar and opercular spaces. Table 2 demonstrates that, in the absence of such an external catalyst, water temperature and/or pH would have to be outside the lethal limits of many aquatic species for uncatalysed CO_2:HCO_3 reactions to occur rapidly enough to have an appreciable effect on water pH within the interlamellar transit time for gill water (0.10 - 0.15 sec, Randall et al. 1982).

What is the pH of water adjacent to the gill surface? One would predict, given the slow rate of uncatalysed CO_2 reactions, that CO_2 excretion would have little, if any, effect on boundary layer pH. Of course, CO_2 and HCO_3 are not the only acid-base relevant molecules transfered across the gills. Movements of NH_3, NH_4^+, H^+ and OH^-, amongst others, across the gills will also influence boundary layer pH. The net effect of all of these transfer processes on boundary layer pH will depend upon the direction and magnitude of the fluxes, the interlamellar transit time of gill water, the uncatalysed rates of CO_2:HCO_3^- and NH_3:NH_4^+ interconversions, the buffering capacity of the ambient water, and the volume of the boundary layer. As such, boundary layer pH must be a function of ambient water quality (temperature, pH, ionic strength and composition), ventilation rate and volume, and the mode of ventilation (buccal pumping versus ram ventilation). The mass ratio for branchial excretion of CO_2:NH_3:H^+:HCO_3^- in rainbow trout at rest in freshwater is about 19:3:3:1, assuming (a) a ratio for CO_2:HCO_3^- excretion of 19:1 (Cameron and Polhemus 1974), (b) a ratio for total CO_2 production to total ammonia production of 8:1 at a steady state respiratory quotient of 0.8 (Heisler 1984), (c) a one-for-one branchial exchange of Na^+/H^+ and HCO_3^-/Cl^- with the rate of Na^+ influx being about three times that of Cl^- (Eddy 1982), and (d) that simple diffusion of NH_3 accounts for ammonia excretion (Cameron and Heisler 1983). Protonation of excreted NH_3 ($NH_3 + H^+ = NH_4^+$) within the boundary layer will ameliorate acidifying effects of H^+ excretion and CO_2 hydration; this protonation reaction occurs rapidly enough to be complete within the interlamellar transit time of gill water. The precise effect of gill fluxes on boundary layer pH will be extremely temporal, however, since the magnitude and even the direction of these fluxes are sensitive to many biotic and abiotic variables including exercise, respiratory acidosis, and water ammonia levels and salinity (Eddy 1982, Heisler 1984, Cameron and Heisler 1983). Moreover, enhanced interlamellar turbulence during periods of rapid or vigorous buccal pumping may eradicate gill boundary layers and directly expose the gill surface to bulk water. Considering the sensitivity limits of research pH meters (± 0.001 units) and the accuracy of precision calibration buffers (± 0.005 units), measurement of bulk water pH is probably the best possible indicator of the pH of water adjacent to the gill surface. Models that attempt to explain the observed effects of pH on the aquatic toxicity of compounds, such as ammonia (Lloyd and Herbert 1960, Szumski et al. 1982), in terms of a large CO_2-induced acidification of the gill boundary layer must be regarded as being over-simplified to the point of being erroneous.

ACKNOWLEDGMENTS

Dr. D.J. Randall is thanked for his intellectual and material support of this work. Financial support was provided by a University of British Columbia Graduate Fellowship to the author. The work described in this paper was not funded by the U.S. Environmental Protection Agency and therefore the contents do not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Broderius, S.J., L.L. Smith Jr., and D.T. Lind. 1977. Relative toxicity of free cyanide and dissolved sulfide forms to the fathead minnow (Pimephales promelas). J. Fish Res. Board Can. 34, 2323-2332.

Boutilier, R.G., T.A. Heming, and G.K. Iwama. 1984. Physiochemical parameters for use in fish respiratory physiology. pg. 403-430. In Fish physiology, volume X, in W.S. Hoar and D.J. Randall, editors. Academic Press, New York, New York.

Buysman, J.R., and F.T. Koide. 1971. Ion concentration profile normal to cell membrane. J. Theor. Biol. 32: 1-23.

Cameron, J.N., and N. Heisler. 1983. Studies of ammonia in rainbow trout: Physico-chemical parameters, acid-base behaviour and respiratory clearance. J. Exp. Biol. 105, 107-125.

Cameron, J.N., and J.A. Polhemus. 1974. Theory of CO_2 exchange in trout gills. J. Exp. Biol. 60, 183-194.

Dimberg, K., L.B. Hoglund, P.G. Knutsson, and Y. Ridderstrale. 1981. Histochemical localization of carbonic anhydrase in gill lamellae from young salmon (Salmo salar L.) adapted to fresh and saltwater. Acta. Physiol. Scand. 112, 218-220.

Eddy, F.B. 1982. Osmotic and ionic regulation in captive fish with particular reference to salmonids. Comp. Biochem. Physiol. 73B, p. 125-141.

Emerson, K., R.C. Russo, R.G. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J. Fish. Res. Board Can. 32: 2379-2383.

Haswell, M.S., D.J. Randall, and S.F. Perry. 1980. Fish gill carbonic anhydrase: Acid-base regulation or salt transport? Amer. J. Physiol. 238, R240-R245.

Heming, T.A. 1984. The role of fish erythrocytes in transport and excretion of carbon dioxide. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

Heisler, N. 1982. Transepithelial ion transfer processes as mechanisms for fish acid-base regulation in hypercapnia and lactacidosis. Can. J. Zool. 60: 1108-1122.

Heisler, N. 1984. Acid base regulation in fishes. pg. 315-407. In Fish physiology, volume X, W.S. Hoar and D.J. Randall, editors. Academic Press, New York, New York.

Jacobs, M.H. 1940. Some aspects of cell permeability to weak electrolytes. Cold Spring Harbour Sym. Quant. Biol. 8, 30-39.

Kern, D.M. 1960. The hydration of carbon dioxide. J. Chem. Educ. 37, 14-23.

Lacy, E.R. 1983. Histochemical and biochemical studies of carbonic anhydrase in the opercular epithelium of the euryhaline teleost, Fundulus heteroclitus. Amer. J. Anatomy 166, 19-39.

Lloyd, R., and D.W.M. Herbert. 1960. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (Salmo gairdneri Richardson). Ann. Appl. Biol. 48, 399-404.

Perry, S.F., and T.A. Heming. 1981. Blood ionic and acid-base status in rainbow trout (Salmo gairdneri) following rapid transfer from freshwater to seawater: Effect of pseudobranch denervation. Can. J. Zool. 59, 1126-1132.

Randall, D.J., S.F. Perry, and T.A. Heming. 1982. Gas transfer and acid/base regulation in salmonids. Comp. Biochem. Physiol. 73B, 93-103.

Roseboom, D.P. 1983. Discussion of: Evaluation of EPA un-ionized ammonia toxicity criteria. J. Water Poll. Control Fed. 55: 420-421.

Szumski, D.S., D.A. Barton, H.D. Putnam, and R.C. Polta. 1982. Evaluation of EPA un-ionized ammonia toxicity criteria. J. Water Poll. Control Fed. 54, 281-291.

Tabata, K. 1962. Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide. Bull. Tokai Reg. Fish Res. Lab. 34: 67-74 (Transl. from Japanese).

Thurston, R.V., R.C. Russo, and G.A. Vinogradov. 1981. Ammonia toxicity to fishes. Effect on pH on the toxicity of the un-ionized ammonia species. Environ. Sci. Techn. 15, 837-840.

Tomasso, J.R., C.A. Goudie, B.A. Simco, and K.B. Davis. 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Trans. Amer. Fish. Soc. 109: 229-234.

U.S. EPA (U.S. Environmental Protection Agency). 1977. Quality criteria for water. Office of Water and Hazardous Materials, U.S. Environmental Protection Agency, Washington, D.C.

USE OF INDICATORS OF FUNCTIONAL STATE OF BIOTA IN BIOLOGICAL MONITORING OF SURFACE WATERS

by

V.A. Bryzgalo, L.S. Fedorova, T.A. Khoruzhaya,
L.S. Kosmenko and L.P. Sokolova[1]

ABSTRACT

Discussed is the use of various functional characteristics of aquatic ecosystems in the biomonitoring of surface waters. A study of the system as a whole with an assessment of the interaction between the elements of the system and between the system and the surrounding environment has provided an opportunity to assess the intensity and direction of the basic processes involved in the transformation of matter and energy in the communities. These processes include photosynthesis, fixation of CO_2, consumption of organic matter, etc.

Particular attention was given to evaluating the information provided by the individual physiological and biochemical indicators for the state of the biota. Research was carried out on aquatic objects under varying conditions of thropogenic stress. In addition the main criteria were established for assessing the trend and intensity of production-destruction processes.

INTRODUCTION AND DISCUSSION

Biological monitoring of surface waters is done in the USSR by the hydrobiological network of the National Service for Observation and Monitoring of the Environment (OGSNK) on three levels. impact, regional, and background. At the present time, methods used for this purpose are based on traditional hydrobiological observations of systematic identification of species, as well as saprobiological analysis (Anon. 1983). Studies are made of total number of organisms, biomass, number of species, marker significance, etc.

---

[1] Hydrochemical Institute, Rustov-on-Don, USSR

Such an approach makes it possible to assess the entire ecosystem of a body of water on the basis of data concerning the condition of phytoplankton, zooplankton, bacterioplankton, periphyton, zoobenthos, and macrophytes. Saprobic indexes, Vudiviss's biotic index and several other procedures, which permit comparing both different parts of reservoirs to one another and different water systems, are used for a formalized evaluation of these hydrobiocenoses.

All these methods and approaches make it possible to describe the structure of an ecosystem, but furnish virtually no information about its function. Among the functional methods recommended for use in the OGSNK network, we should include those for determination of primary production and destruction of organic matter of phytoplankton, bacterioplankton and zooplankton, as well as pigments of phytoplankton, that are used by different network hydrobiological laboratories.

One of the reasons for insufficient use of functional indicators in the system of biological labeling of surface water is the highly dynamic nature of aquatic communities, as well as the wide spatial and time heterogeneity of their characteristics, and significant seasonal fluctuations in development of the different elements of an ecosystem. At the same time, the need to use the functional approach for biological monitoring of surface waters is growing increasingly pressing and it is dictated by a number of factors. The very definition of the concept of "norm" as a zone of optimum function of a system requires use primarily of functional characteristics to assess the ecological state of water systems. Functional parameters are also important from the standpoint of the basic water protection tasks, because they make it possible to turn to evaluation of the process of formation of water quality, the capacity of hydrobiocenoses to maintain stability of quality characteristics of water.

The speed of functional methods, as compared to structural ones, is one of the appreciable advantages of the former particularly in effective monitoring (Israel' 1983).

Successful use of functional parameters can be made only if distinct correlations are established between changes in them and changes in the corresponding parameters of the environment caused by exogenous factors. However, even with this formulation of the problem, a number of difficulties arise. For example, it is quite obvious that direct measurement of results of the dose-effect reaction in a water system, particularly in studies of plankton communities, is impossible at the present time. As for model experiments, it is hardly valid to extrapolate data to natural water ecosystems due to the absence of more or less distinct ecological criteria of similarity. Finally, use of the probabilistic statistical approach requires comprehensive and detailed analysis of aquatic ecosystems, which is hardly expedient for economic considerations.

One of the routes of solving this problem is to find methods of evaluating the results of population (or even community) activity, provided there is substantial reduction of spatial resolution of information. This becomes feasible when studies are planned in such a way that the water system is considered, on the one hand, as a unique ecosystem and, on the other hand, as separate ecosystems in the form of contrasting water masses characterized by specific physicochemical conditions and populations, on the assumption that there is relatively little interaction between them.

It is possible to implement such an approach if we proceed from the following. Different physicochemical conditions determine formation of different ecosystems. This permits selection of representatives for a given type of study of a region, which reduces spatial resolution of the study and increases time resolution. And, using structural and functional parameters, one can gain an idea about the extent and direction of main processes of transformation of matter and energy in aquatic ecosystems, and assess the informativeness of biochemical parameters in biomonitoring of water quality.

Properly validated conclusions about the ecological state of water systems are difficult to derive because of the lack of unified and adequate methodological approaches for quantitative description of processes that are of interest from the standpoint of trophodynamics. To date, quite a few functional characteristics of state of marine ecosystems have been proposed and developed. A significant number of studies have dealt with investigation of different functional parameters of phytoplankton.

As far back as the turn of the century, hydrobiologists began to view phytoplankton as the main producer of organic matter--and the only one in vast expanses of ocean--on the basis of which the entire diversity of aquatic life is formed. This was the reason for the heightened interest in investigating not only the species composition of phytoplankton and its quantitative distribution, but various parameters characterizing its function. We are referring, first of all, to output, i.e., rate of production of organic matter by phytoplankton. To determine this, one generally uses physiological methods based on comparing the results of determining intensity of photosynthesis (production) and respiration (destruction) of communities according to oxygen balance (Federov 1979).

In spite of the fact that the oxygen method used in most cases has some flaws, as well as of the dissatisfaction of many researchers with obtained results, it became possible to formulate tasks such as determination of productivity of autotrophic and heterotrophic organisms. It is expressly in trying to assess their contribution to overall production of an aquatic ecosystem that more sensitive radioactive carbon methods were developed for measuring phytoplankton output. Functional methods, which characterize the state and activity of phytoplankton and, in particular, different parameters of intensity of photosynthesis, underwent the greatest development. We should mention here fluorescence and luminescence techniques, which are highly sensitive and permit use of rather simple equipment and instruments (Sirenko 1983).

We should also include among the functional methods those used to investigate processes of transformation and utilization of organic matter by hydrobionts. The mechanisms of these processes and conditions that make them efficient are being studied intensively, although we are still far from comprehending the key aspects of the problem (Pursons et al. 1982). Of definite interest are studies of heterotrophic activity of aquatic cenoses both to assess their role in forming the qualitative composition of natural waters and as an informative integral indicator of the conditon and stablity of aquatic ecosystems.

Interest in studying bacterioplankton as related to water systems differing in trophism was prompted by the substantial contribution of this community to processes of utilization of organic matter. Measurement of uptake of glucose and comparing it to the total quantity of ATP-containing bacteria, concentration of chlorophyll a, changes in pH, and levels of oxygen, carbon dioxide, nitrogen and phosphorus made it possible to define the trophic status of 21 lakes in a mountain province of New Zealand (Spenser 1978). Apparently, one can define the time and space parameters of distribution of pollutants according to rate of uptake of organic matter. Thus, Pearl and Goldman (1972) obtained encouraging results using the rate of acetate uptake as an indicator of the condition of the lake's water masses.

In recent years, there has been increasing discussion of the possibility of using kinetic parameters of uptake of organic matter by natural waters based on examination of dynamics of assimilation of ^{14}C from water samples with addition of labeled organic compound (Wright and Hobbic 1966).

By using structural and functional methods of studying aquatic communities, one can gain an idea about the extent and direction of basic processes in aquatic ecosystems. This is possible thanks to the fact that, by studying the dynamics of functional parameters against the background of change in structure of communities, we obtain a description of processes--in particular, of photosynthesis, fixation of CO_2, uptake of organic matter, etc. It is expedient to use various coefficients--for example, the ratio of destruction to biomass (D/B) and production to biomass (P/B).

Such estimates bring us to a description of extent of thermodynamic organization of the ecosystem or hydrobiocenosis in question (Odum 1975). Some results of using these coefficients for biomonitoring were recently summarized in an article by Vernichenko and Starko (1982). It should be stressed that a more or less distinct range of changes in these coefficients has not yet been defined due to insufficient development of both the methods of measuring primary production and destruction, and biomass. In the OGSNK network, the P/D coefficient is used to determine the intensity of production-destruction processes. The range of changes in this indicator has been studied more, it is used, for example, by specialists at the IGB of the Ukrainian Academy of Sciences in a plan for a system of integrated evaluation of quality of surface waters (Zhukinskiy et al. 1978).

Primary production and destruction, as well as biomass levels, are very dynamic and subject to substantial time and space changes, they depend on seasonal fluctuations, hydrological conditions in the reservoir and other factors. All this makes it considerably more complicated to use them for biomonitoring. The validity of this statement is well-illustrated in the studies we conducted on reservoirs differing in trophism using the above-described approaches.

The purpose of the study included demonstration of the main structural and functional indicators of the state of the biota, which are related to formation of quality of natural waters as related to different anthropogenic loads, as well as determination of the main criteria for assessing the direction and intensity of production and destruction processes.

The studies were conducted in a "conditionally pure" eutrophic reservoir, with predominantly natural eutrophication, and an oligotrophic one with an anthropogenic load, during a period of natural alternation for the reservoir of dominant groups of algae, starting with diatoms and ending with blue-green algae, covering the entire period of development of the latter (from 0 to 98% of total phytoplankton biomass).

In the period of phytoplankton development we studied, there were three peak levels of total biomass, one of which was attributable to development of diatoms (7 July), and the two others to biomass of blue-green algae (25 July and 15-17 August). The natural replacement of dominants (diatoms--blue-green algae) occurs in active competition between green and blue-green algae, and it is notable for drastic fluctuations in biomass level and photosynthetic activity per unit phytoplankton biomass (P/B coefficient) (Table 1). Development of blue-green algae is associated with relatively uniform increase in total biomass and production of phytoplankton. The P/B coefficient also rises, and during the period of drastic dominance of blue-green algae, it changes by no more than 4 times. The changes in primary production are characterized by two maximums, one of which corresponds to the period of diatom "blooming" (7 July), and the second, which is more significant, corresponds to the period of marked "blooming" of blue-green algae (15-19 August).

Biologically, production is the most important function in the structure of the community, when the structure is stable, the functional parameters, in particular P/B, remain constant. Any change in structure of the community leads to change in production rate. We found that the increase in phytoplankton biomass is faster at the early stage of "blooming" than the increase in production. For this reason, there is decline of photosynthetic activity per unit phytoplankton biomass (P/B coefficient).

While we can judge the status of an ecosystem as a whole from the P/B ratio, the ratio of phytoplankton production to its population size (P/S) enables us to turn to a description of the condition of cells of individual specimens, which is determined primarily by presence of nutrients in their environment.

Evidently, this ratio could indicate, indirectly, the purpose of phytoplankton activity: intensive division and increase in number or accumulation of nutrients in the cell, i.e, increase of its biomass. Indeed, during the period of diatom and green algae blooming, when activity is directed toward retaining their populations, there is a maximal P_p/S ratio $(0.2-0.8)\cdot10^{-3}$. Under favorable ambient conditions, the dominant blue-green algae expend a significant part of the newly formed organic matter on cell division and increase in population size. The P_p/S ratio is then smaller by a factor of 10 $(0.2-0.3)\cdot10^{-3}$.

TABLE 1. CHARACTERISTICS OF PHYTOPLANKTON (p) ACTIVITY ACCORDING TO SOME PARAMETERS

Date of taking sample	Total number (S), thousands cells/mℓ	Biomass (B_p) mg C/ℓ	Production (P_p), mg C/(ℓ day)	P_p/B_p, mg C/(mg·day)	P_p/S, μg C/(cells·day)	Heterotrophic pyruvate uptake, μg C/(ℓ·day)
7 Jul	2.0	0.145	1.60	11.0	0.80 10^{-3}	--
9 Jul	1.96	0.047	0.30	6.0	0.15 10^{-3}	0.16
11 Jul	2.00	0.045	0.47	10.0	0.24 10^{-3}	0
14 Jul	18.70	0.470	0.49	1.0	0.03 10^{-3}	--
25 Jul	14.55	1.100	0.32	0.3	0.02 10^{-3}	0.12
30 Jul	50.60	0.790	1.25	1.6	0.02 10^{-3}	4.74
10 Aug	82.60	0.760	2.38	3.0	0.03 10^{-3}	0
12 Aug	95.00	1.140	2.88	2.5	0.03 10^{-3}	5.3
15 Aug	493.00	3.600	29.30	8.0	0.06 10^{-3}	14.5
19 Aug	706.00	5.060	13.10	2.6	0.02 10^{-3}	0
22 Aug	8.50	0.160	1.16	7.0	0.13 10^{-3}	0

The rate of pyruvate uptake by blue-green algae reached 5-15 μg C/(ℓ·day).

Hydrochemical studies revealed that, during the period of prevalence of blue-green algae, levels of mineral forms of nitrogen and phosphorus were favorable for their development, and the C:N:P ratio was optimum. However, the results of measuring heterotrophic activity of algae (assay of phytoplankton uptake of ^{14}C-pyruvate) revealed that, even without a shortage of mineral nutrients, blue-green algae at the stage of maximum photosynthetic activity are capable of utilizing readily available organic substances.

As we know, intensity of metabolism, i.e, ratio of respiration to biomass (R/B) determines the extent of thermodynamic organization (Wright and Hobbie 1966), which could characterize the reaction of the ecosystem to an exogenous factor. Most often, one uses the P/B ratio, rather than R/B, and the former expresses the increment of production per unit initial or mean value. P/B, the coefficient or its reciprocal B/P-coefficient of turnover of biomass--are considered by many researchers to be the most informative parameters characterizing the production capacities of a given species or entire community.

During the period of intensive phytoplankton blooming, bacterioplankton reacts to its environment by increasing its functional activity (Table 2). Bacterioplankton production reaches a maximum during the period of maximum development of blue-green algae (15-19 August). However, if we calculate averaged activity per cell (ratio of P to total population size), the result will indicate that, in spite of the large number of cells, bacterioplankton is in a relatively depressed state during the period of dominance of blue-green algae. Activity drops from $6 \cdot 10^{-9}$ μg C/day to $0.76 \cdot 10^{-9}$ μg C/day.

The ratio of heterotrophic ^{14}C-pyruvate heterotrophic uptake to bacterial biomass production, as determined from dark assimilation of CO_2, can also serve as an indirect indicator of the state of bacterioplankton. The higher the activity of bacterial cells, the closer the ratio is to 1.

These studies enabled us to conclude that neither total population size nor biomass, nor even such functional parameters as production of phytoplankton and bacterioplankton could yield sufficient information about the state of the natural population. It is only by turning to a description of activity of the population unit (one cell) that we come close to characterizing the actual response of organisms to a change in their environment, in both the case of its natural change at the early stage of the eutrophication process and in the case of "severe" anthropogenic pollution. The seeming "wellbeing" of the ecosystem, which can be assessed by the structural organization of the cenosis, conceals appreciable changes in condition of organisms in different trophic chains and the population as a whole (specific activity, rate of biomass turnover, doubling time, intensity of respiration, coefficient of energy metabolism, etc.). These changes in status of the ecosystem can be detected only if studies are made with sufficient spatial and time resolution.

Approaches based on evaluation of different physiological and biochemical parameters are among promising methods that have not yet been

TABLE 2. CHARACTERISTICS OF BACTERIOPLANKTON ACTIVITY ACCORDING TO SOME FUNCTIONAL PARAMETERS

Date of Taking Sample	Total number (S), millions cells/mℓ	Production (μg C/(ℓ·day), as determined by uptake of		P_g/P_h	Specific production P_g/S, μg C/(cells·day)
		^{14}C-carbonate (P_h)	^{14}C-pyruvate (P_g)		
9 Jul	0.54	2	1.31	0.65	2.4 10^{-9}
11 Jul	0.79	8	4.68	0.58	6.0 10^{-9}
14 Jul	2.01	8	8.17	1.02	4.0 10^{-9}
25 Jul	0.94	4	2.87	0.71	3.0 10^{-9}
29 Jul	13.20	40	17.30	0.43	1.3 10^{-9}
30 Jul	17.60	10	13.48	1.35	0.76 10^{-9}
31 Jul	4.60	17	4.37	0.26	0.95 10^{-9}
10 Aug	--	6	1.50	0.26	--
12 Aug	--	35	8.42	0.24	--
15 Aug	--	44	32.80	0.75	--
19 Aug	--	89	22.46	0.25	--
21 Aug	1.05	5	0.82	0.16	0.78 10^{-9}
22 Aug	2.90	12	3.80	0.32	1.30 10^{-9}

sufficiently developed, which reflect the functional state of aquatic ecosystems. In our opinion, biomonitoring methods involving use of parameters of enzymatic activity of both individual hydrobionts and populations, and even aquatic communities merit special attention. Proceeding from the key theses of biology to the effect that a reaction to environmental changes is manifested primarily by change in rate and nature of enzymatic processes, it can be assumed a priori that the level of enzyme activity could be used to assess water quality. The response of an enzyme system characterizes the state of aquatic organisms when there are fluctuations in physicochemical parameters of the environment, while the functional reserves reflect the degree of adaptation to them.

This approach is not without some flaws. On the one hand, some enzymatic reactions are "universal" in a sense and provide for basic metabolic processes over a rather wide range of fluctuations of conditions in the aquatic environment. On the other hand, some biochemical parameters are "specific," i.e., they reflect a response to only a specific type of pollution.

Thus, use of biochemical parameters, including enzymatic ones, requires a validated choice of methods that are adequate to the purpose of observation, as well as determination of the range of changes in a given enzymatic function in hydrobionts that are not exposed to a factor, i.e., definition of the reaction "norm." The difficulty of this problem as it applies to biomonitoring of the quality of natural waters has already been stressed (Braginskiy 1981).

We used the biorhythmological approach in studies of enzymatic activity of naturally occurring populations of mollusks exposed to the chronic effect of sewage from the paper and pulp industry. It was established that, in gastropod mollusks taken from different biohydrocenoses, the curves of circadian activity of enzymes--esterases, alkaline phosphatase, succinate, malate and lactate dehydrogenases--differed. Depending on the role of the tested enzymatic systems, we observed prevalence of "diurnal" or "nocturnal" activity.

To compare these data in mollusks taken from different sections of the reservoir, we calculated the "parameter of circadian adaptability" (PCA) for each enzyme. We found that PCA was positive for virtually all enzymes in mollusks inhabiting a conditionally pure zone. In mollusks taken from a part of the reservoir subject to the chronic effect of sewage, PCA was negative for all enzymes. Mollusks living in zones differing in degree of pollution differed not only in biorhythms of enzymatic activity, but response to an additional burden-- brief change in temperature. Thus, with exposure to change in temperature, the mollusks of the "conditionally pure zone" showed some decline in enzyme activity, while the nature of the circadian rhythm did not change appreciably. In mollusks from the zone subject to the effect of sewage, the circadian rhythms of all enzymes studied changed appreciably, and their activity dropped drastically. Moreover, we observed much faster restoration of initial rhythms in mollusks of the control zone than those exposed to the influence of sewage.

Thus, it should be concluded that organization of the circadian rhythm of enzyme activity in naturally occurring populations of mollusks can be used as a characteristic of functional state of an organism, its capacity for adaptation under altered physicochemical environmental conditions, as well as extent of effect of environmental factors. Apparently, considering the significant fluctuations of hydrobionts' biochemical parameters, which are a typical feature and reflect adaptation to the aquatic environment, the biorhythmological approach can be recommended for studies on different levels of organization--cellular, tissular, organic, organism, population, etc.

At the present stage of development of scientific methodological procedures for evaluating the ecological status of the naturally occurring water environment, an integrated approach is needed for analysis of suitability of physiological and biochemical parameters for biomonitoring of surface waters. It should be noted that most of the methods based on physiological and biochemical parameters are referable to biological tests, and they were developed primarily to assess the toxicity of sewage or chemical compounds (when setting maximum permissible concentrations). Trial of these methods in naturally occurring waters requires concurrent investigation not only of hydrobiological parameters characterizing the structure of the water ecosystem, but of hydrological-hydrochemical parameters. The latter is particularly necessary when investigating physiological and biochemical parameters on the level of populations and aquatic communities, due to the complexity and diversity of links between both individual trophic levels and between the biota and environment, as well as the very dynamic nature of these links.

Viewing enzymatic activity as a function of interaction of an organism with the environment, which changes in the time parameter of development of the ecosystem and trophodynamic processes, we analyzed the informativeness of enzymatic parameters of naturally occurring seston communities in waters subject to natural eutrophication and anthropogenic influences. In these studies we used the following parameters: species composition, total number and biomass of bacterioplankton, phytoplankton and zooplankton, intensity of production and destruction processes, activity of alkaline phosphatase and total esterases of seston, levels of biogenous elements (total, nitrate, and nitrite nitrogen, ammonia nitrogen, total and mineral phosphorus), and levels of oxygen, hydrocarbonates and pH.

It was established that the absolute levels of enzyme activity are low in an aquatic ecosystem that is not exposed to any significant anthopogenic factors, while potential activity (A_p) is 3 to 5 times higher than the level observed (A_o). Distinct seasonal dynamics of changes in enzyme activity can be observed. In situations related to drastic changes in environmental conditions (pollution, algal "blooming," etc.), absolute enzyme activity levels rise and there is change in seasonal cycle of enzyme activity, as well as in A_p:A_o. Determination was made of the range of fluctuations of enzyme activity during the period of blooming of blue-green algae with exposure to sewage from the paper and pulp industry, as well as with salt pollution. It was shown that the reaction of seston with regard to enzyme activity during natural eutrophication of the reservoir is described by a curve that is synchronized with the developmental phases of bacteria and algae, as well as changes in proportion of biogenous elements contained in the environment. With decline of mineral phosphorus level in water, there is increase in esterase activity and the enzyme activity ratio (esterase:alkaline phosphatase) increases by a factor of 10. In the case of anthropogenic influences, enzyme activity of seston increases drastically, which makes it possible to differentiate water zones in the region of waste dumping according to extent of pollution.

In conclusion, we should like to emphasize that there are several tasks that are of prime importance in the problem of biomonitoring of natural waters. As a result of hydrobiological observations pursued in the system of state supervision, information for a period of many years has been accumulated. This information is presently being systematized in order to find the parameters that reflect the basic trends of change, mainly in structural organization of aquatic communities as a function of extent and nature of anthropogenic burden on a reservoir's ecosystem as a whole.

Analysis of the accumulated information, with consideration of results of scientific studies dealing with biomonitoring of natural waters, including functional parameters, will enable us to undertake development of the methodology for assessing the ecological status of water systems and developing the optimum variant of routine and efficient biomonitoring.

Introduction of new methods, which permit evaluation of functional changes in the ecosystem of a body of water, offers vast opportunities for both efficient monitoring and forecasting the state of the ecosystem and water quality. Unification and standardization of both approaches and procedures, as well as methods as a whole, is a mandatory prerequisite for successful use of these methods.

BIBLIOGRAPHY

Anon. 1983. Rukovodstvo po metodam gidrobiologicheskogo analiza poverkhnostnykh vod i donnykh otlozheniy (Manual of Methods for Hydrobiological Analysis of Surface Waters and Bottom Deposits). Leningrad, USSR. 239 p.

Braginskiy, L.P. 1981. Theoretical aspects of the problem of normal and pathology in aquatic ecotoxicology. In Teoreticheskiye voprosy vodnoy toksikologii (Theoretical Problems of Aquatic Toxicology). Leningrad, USSR. pp. 29-40.

Fedorov, V.D. 1979. O metodakh izucheniya fitoplanktona i yego aktivnosti (Methods of Studying Phytoplankton and Its Activity). Moscow, USSR. 166 p.

Israel', Yu.A. 1983. Ekologiya i kontrol' sostoyaniya prirodnoy sredy (Ecology and Inspection of the Environment). Leningrad, USSR. 376 p.

Odum, J. 1975. Fundamentals of ecology. Moscow, USSR. 740 p.

Pearl, H.W. and C.R. Goldman. 1972. Heterotrophic assays in the detection of water masses of Lake Tahoe, California. Limnol. and Oceanogr. 17(1):145-148.

Pursons, T.R., M. Tachakashi, and B. Chargrive. 1982. Biological oceanography. Moscow, USSR. 432 p.

Sirenko, A.A. 1983. Curve of vertical distribution of chrlorophyll in a body of water as an indicator for integral evaluation of correlation between production and destruction processes. In Obobshchennyye pokazateli kachestva vod--83. Prakticheskiye voproshy biotestirovaniya i bioindikatsii. Tezisy dokl. (Summaries of Papers Delivered on Overall Indicators of Water Quality--1983. Practical Problems of Biological Testing and Biological Monitoring). Chernogolovka, USSR. pp. 124-126.

Spenser, M.J. 1978. The trophic status of 21 lakes of Mountain Province in New Zealand. N.Z. Mar. and Freshwater Res. 12(4):415-427.

Vernichenko, A.A. and N.V. Starko. 1982. Prospects of using functional indicators in the system of monitoring aquatic ecosystems. In Kontrol' kachestva prirodnykh i stochnykh vod (Monitoring Quality of Natural and Run-Off Waters). Kharkov, USSR. pp. 14-20.

Wright, R.T. and J.G. Hobbic. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology. 47(3):447-464.

Zhukinskiy, V.N., O.P. Oksiyuk, G.N. Oleynik, and S.I. Kosheleva. 1978. Plan for a system of integral evaluation to quality of surface waters, Vodnyye Resursy. (3):83-93.

LONG RANGE TRANSPORT OF TOXIC ORGANIC CONTAMINANTS TO THE NORTH AMERICAN GREAT LAKES

by

W.R. Swain[1]
M.D. Mullin[2]
J.C. Filkins[2]

ABSTRACT

Studies of persistent organic contaminants made for the Upper Lakes Reference Group of the International Joint Commission in 1974-1976 indicated increased levels of organic contamination in the flesh of fish taken from the vicinity of Isle Royale in Lake Superior. These findings led to a preliminary study of polychlorinated biphenyl (PCB) compounds in atmospheric precipitation deposited in Siskiwit Lake on Isle Royale.

The subsequent studies reported here support the earlier findings but show significant shifts in observed concentrations. Initially, these altered values were thought to be the result of increasing environmental concentrations of PCBs. The use of refined analytical procedures suggests, however, that the apparent changes are a function of the co-occurrence of derivatives of the pesticide Toxaphene within the analytical spectrum of the PCB fraction. A comparison of the older data from Siskiwit Lake with those acquired from contemporary analytical procedures suggests that it is likely that derivatives of Toxaphene may have been enumerated with PCB peaks, thus being inadvertently reported as total PCB. Based on results obtained with high resolution capillary chromatography, apparent increases in concentrations of PCB compounds in 1980 packed column chromatography samples are undoubtedly a function of historic analytical limitation, rather than an absolute change in environmental concentrations of the PCB compounds.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

---

[1] Vakgroep Aquatische Oecologie, Universiteit van Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands

[2] Large Lakes Research Station, U.S. Environmental Protection Agency, Grosse Ile, Michigan 48138 USA

INTRODUCTION

In 1974, studies of the accumulation of selected persistent organic residues in the fish species of the Lake Superior ecosystem were initiated under the auspices of the International Joint Commission's Upper Lakes Reference Group. The findings of these studies indicated substantially increased levels of halogenated organic substances in Lake Superior fish near Isle Royal, particularly in lean lake trout, Salvelinus namaycush, and in fat lake trout, Salvelinus namaycush siscowet.

As a check on contaminant levels in Lake Superior fish, a control site, Siskiwit Lake on Isle Royale, was established. This deep, cold, oligotrophic lake contained indigenous species of fish similar to those in Lake Superior, allowing direct comparison. The island, and hence the lake, was remote from inhabited areas, thus being well removed from the industrial and cultural influences of man's activities. Surprisingly, however, the values for several organic contaminants in the flesh of fish from Siskiwit Lake were significantly higher than corresponding values in fish from Lake Superior. Polychlorinated biphenyl (PCB) compounds were nearly double the Lake Superior mean value, and p,p-DDE showed a more than 10-fold increase in Siskiwit Lake. These findings led to a preliminary study of the transport of PCB compounds in atmospheric precipitation. For a more complete summary of this work, the reader is referred to Swain (1978).

The present study was undertaken as a continuation of the earlier effort, with particular interest in the changes in residue levels in the fish population of this landlocked, remote island lake.

GEOGRAPHICAL SETTING

Isle Royale is a rock-strewn conglomerate of a central island mass with associated irregular reefs. It lies 27.4 km from the closest shoreward margin of Lake Superior at Thunder Cape, Ontario, but it is more than 50 km to Thunder Bay, Ontario, the nearest major population center (Figure 1).

Isle Royale (Figure 2) has an extreme length of 70.8 km, lying on a southwest-northeast axis. Its maximum width, 14.5 km, occurs near the southwest end of the island. The highest point in the island is Mount Desor, 242 m above Lake Superior. The entire island structure, including its associated reefs and islets, constitutes Isle Royale National Park, established in 1940. Efforts from that date have been made to preserve the island's native wilderness character. As a result, the only means of access from one part of the island to another is by means of foot trails. Siskiwit Lake, accessible only in this fashion, provided a remote aquatic ecosystem relatively undisturbed by human activity.

Siskiwit Lake lies 0.6 km inland from Lake Superior in a depression of Precambrian Middle Keweenawan flow of the Portage Lakes Volcanics. The entire watershed of the lake also lies in bedrock of this type. The long axis of Siskiwit lake follows the same direction as the long axis of Isle Royale, i.e., northeast-southwest.

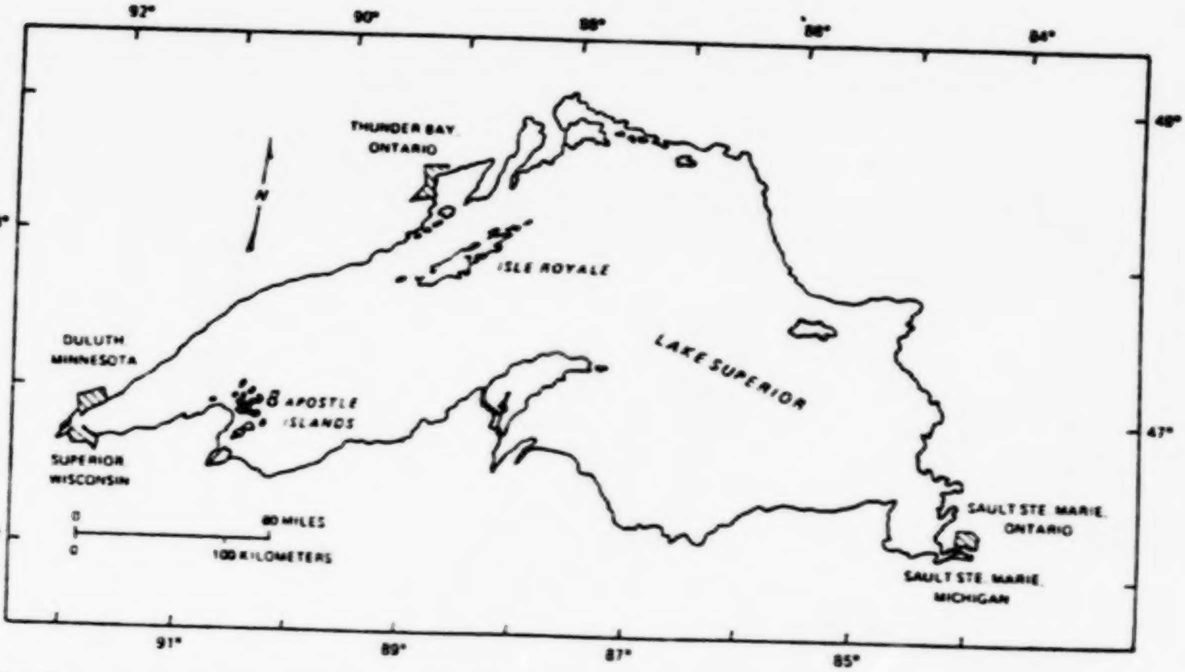

92°
90°
88°
88°
84°
48°
48°
47°
46°
THUNDER BAY
ONTARIO
N
ISLE ROYALE
DULUTH
MINNESOTA
LAKE SUPERIOR
APOSTLE
ISLANDS
SUPERIOR
WISCONSIN
0
80 MILES
0
100 KILOMETERS
SAULT STE MARIE
ONTARIO
SAULT STE MARIE
MICHIGAN
91°
89°
87°
85°

Figure 1. Geographic area of study in Lake Superior.

The length of Siskiwit Lake is 11.10 km, the maximum breadth is 2.25 km, and the mean breadth is 1.40 km (Huber, 1975). The lake area is 15.6 km^2, exclusive of islands. Shoreline development, calculated from the expression $D_L = L/A_o$, is typical of subcircular eliptiform lakes, having a value of 2.66. The elevation of Siskiwit Lake above the mean Lake Superior level is 17.37 m, indicating no direct input from Superior to Siskiwit Lake. Siskiwit Lake drains by a single small stream to Lake Superior, the Siskiwit River. The maximum depth of Siskiwit Lake is 36.58 m.

METHODOLOGY

Sample collection from the remote sites at Siskiwit Lake were made in 1980 from a canoe that was portaged to the site from the Superior shoreline. Fish samples were taken by means of a 350-foot (106.68 m) section of 5.72-cm gill net that was backpacked into Siskiwit Lake and set overnight at a depth of 24.4 to 46.5 m at the location shown in Figure 2. For all collections, the catch was exclusively lake trout (Salvelinus namaycush), whitefish (Coregonus culpeaformis), and bowfin (Amia calva). In each case, only lake trout and whitefish were retained, carried out in a clean mesh bag, eviscerated, iced, and after transport to a populated area, immediately frozen in dry ice for transport to the laboratory.

Snow samples were taken from the undisturbed surface of the lake and were transported to the laboratory in sealed teflon containers. Because of prevailing ambient temperatures, further refrigeration was not necessary.

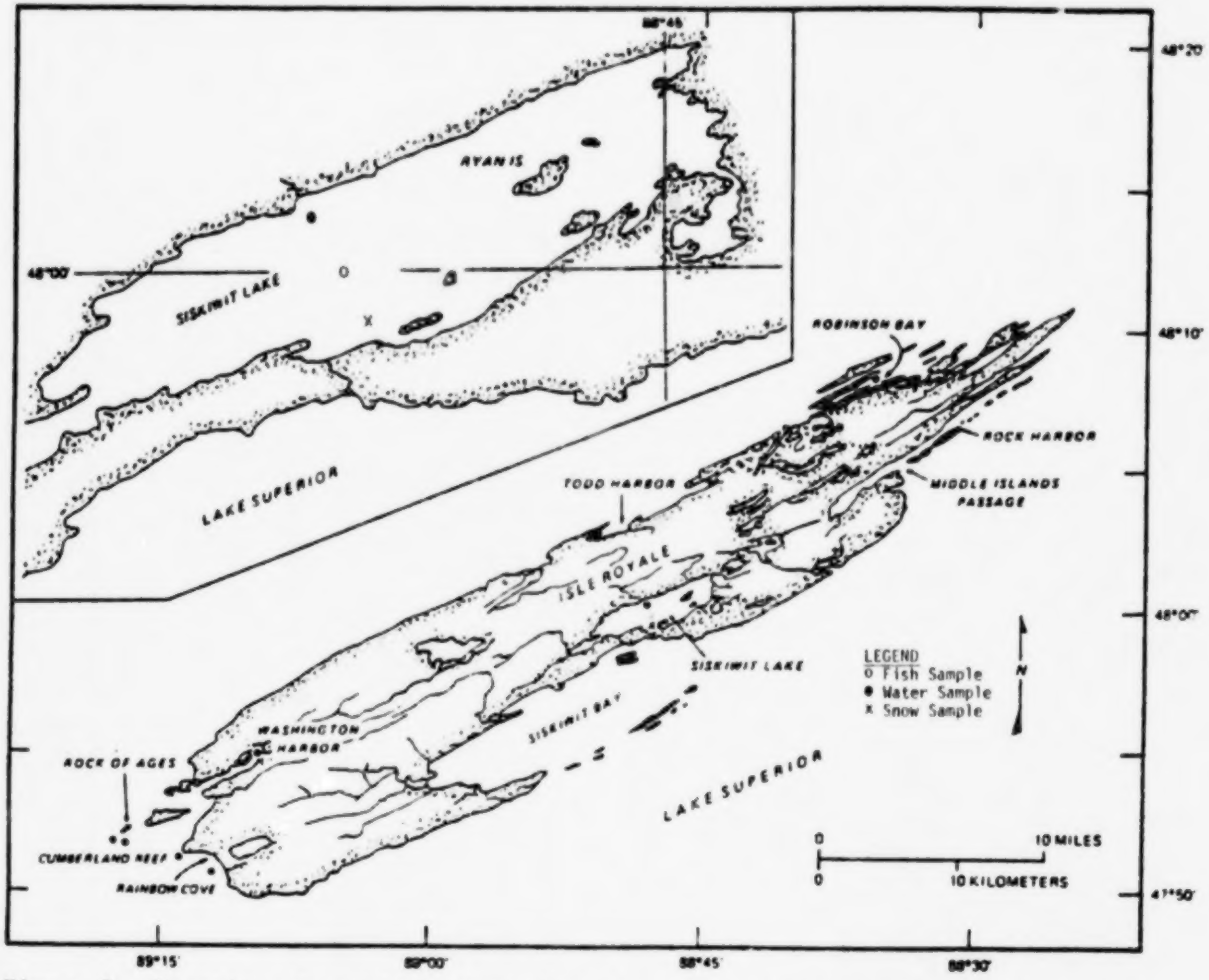

88°45'
RYAN IS
48°00'
SISKIWIT LAKE
LAKE SUPERIOR
ROBINSON BAY
ROCK HARBOR
MIDDLE ISLANDS PASSAGE
TODD HARBOR
ISLE ROYALE
SISKIWIT LAKE
SISKIWIT BAY
WASHINGTON HARBOR
ROCK OF AGES
CUMBERLAND REEF
RAINBOW COVE
LAKE SUPERIOR
LEGEND
o Fish Sample
● Water Sample
x Snow Sample
N
0
10 MILES
0
10 KILOMETERS
48°20'
48°10'
48°0'
47°50'
89°15'
89°00'
88°45'
88°30'

Figure 2. Location of sampling sites for fish, water, and snow in the vicinity of Siskiwit Lake, Isle Royale.

Samples of atmospheric precipitation in the Lake Huron area were taken using both event samplers and bulk collectors lined with thin teflon bags. These bags were suspended within the frame of the bulk collector in such a manner as to minimize evaporation losses from the collected sample. In all but one case, samples were taken from the Michigan side of Lake Huron. The single exception was an August sample taken from the Canadian shore line of Lake Huron below the Bruce Peninsula.

ANALYTICAL PROCEDURES

In an effort to minimize analytical error, samples of fish were submitted to three separate laboratories for analysis. These analytical efforts included the Columbia National Fisheries Laboratory of the U.S. Fish and Wildlife Service at Columbia, Missouri; the Cranbrook Institute of Sciences at Bloomfield Hills, Michigan, and the U.S. Environmental Protection Agency's Large Lakes Research Station at Grosse Ile, Michigan.

Initial packed column gas chromatography studies followed accepted analytical procedure. A detailed description is presented by Swain (1978). When it became apparent that PCB congeners were co-occurring with compounds of Toxaphene, however, subsequent analytical efforts utilized high resolution capillary column chromatography. The procedures associated with this type of analysis are summarized below.

High resolution fused silica capillary gas chromatography was performed on a VARIAN Model 3700 gas chromatograph equipped with a ^{63}NI electron capture detector. A 50-m fused silica column (0.2 mm i.d.) coated with SE-54 (Hewlett-Packard) was used to separate the PCB congeners and the Toxaphene-like compounds. The oven temperature was programmed at a rate of 1.0 °C min^{-1} from 100 to 240 °C. The injector and detector temperatures were 270 and 330 °C, respectively. Sample volume, 6.0 μl, was injected by using an automatic sampler with splitting in the injector (10:1 split ratio, vented from 0.75 to 1.75 min.) The hydrogen carrier gas was held at a constant pressure of 2.25 kg cm^{-2} to give the optimized velocity (μ) at 100 °C of 45 cm s^{-1}.

ANALYSIS

The interpretation of the chromatograms was complicated by the presence, in all samples, of PCBs as well as the polychlorinated camphene (Toxaphene-like) components. The high resolution chromatography used permitted the evaluation of both groups of compounds together without additional separation procedures. There were more than 110 peaks in the PCB reference standard and 39 of the larger peaks in technical Toxaphene were used in the Toxaphene reference standard. Of these approximately 150 different compounds, only about a dozen co-eluted and, of this number, fewer than six were significant. Where co-eluting compounds existed, the following procedure was used: if other PCB peaks in the same time portion of the chromatogram were present and other Toxaphene-like peaks were absent, the peak in question was assumed to be a PCB; conversely, if other PCB peaks in the same time portion of the chromatogram were absent and other Toxaphene-like peaks were present, then the peak in question was assumed to be a Toxaphene-like compound; if both PCB and Toxaphene-like peaks were present, then the peak was excluded from both the PCB and Toxaphene-like calculations.

PCB data were determined by comparing the relative retention times (RRT) of the individual peaks (octachloronaphthalene as internal standard = 1.000) in the chromatogram to those generated by a mixture of Aroclors 1016, 1254 and 1260. If the RRT were within a narrow window of ca 0.0004 RRT units and the height of the peak was proportionate to other PCB peaks in the same time portion of the chromatogram, then the PCB assignment was made and the amount calculated was based on a comparison of the height of the peak in the standard for which the concentration was known) to the height of the peak in the sample.

Toxaphene-like data were calculated differently because of the lack of individual compound standards against which to compare the sample. The 39 peaks in the Toxaphene reference standard all were assigned the same value--the total concentration of the Toxaphene standard--and relative response factors (RRF) for each peak were calculated. Summing these concentrations and dividing by the number of peaks yields the concentration of the solution. Toxaphene-like compounds from the samples were determined in a similar manner. After making qualitative assignments based on the same +/- 0.0004 RRT units exclusion window as used in the PCB determination and comparing the peak height to other Toxaphene-like peaks in the same time portion of the chromatogram to ensure that they were proportionate to the same peaks in the standard, a concentration was calculated for each peak by comparing its height to the height of the same peak in the standard. These concentrations were summed and the sum divided by the number of Toxaphene-like peaks identified in the sample. If fewer than 20 peaks were identified, then the qualitative identification of the peaks as Toxaphene-like was uncertain and reported as questionable.

It should be noted that at the time of preparation of this report, the precise identification of Toxaphene in biologic tissue is still a matter of some analytical discussion. While two laboratories participating in the analytical portion of this study unhesitatingly refer to the material observed as "Toxaphene", a third group is concerned that all of the peaks observed are not entirely co-incidental with pure standards of Toxaphene. This group observes that the sample appears to be "environmentally weathered" as a result of passage through biologic systems. It is their opinion that the substances should be labeled as "chlorinated turpines/camphenes" or as "Toxaphene-like" compounds, suggesting this analytical uncertainty. The latter, more conservative approach has been used for this report.

RESULTS AND DISCUSSION

In 1976, a series of four fresh snow samples was collected, melted, extracted, and analyzed from the Isle Royale site (Figure 2). PCB compounds were expressed as an aggregate of Aroclor 1254, the reference mixture that Veith et al. (1977) reported as most resembling mixtures observed in Lake Superior. This Aroclor mixture was used as an index of airborne movements of persistent organic compounds, and the levels observed at Siskiwit Lake were compared with values at Duluth, Minnesota. The mean value for PCB compounds observed in precipitation samples from the Duluth, Minnesota, metropolitan area was 50.0 ng/l, whereas the mean value from Siskiwit Lake was nearly five times greater, 230.0 ng/l (Swain, 1978). Similar results were reported by Murphy (1976) and by Murphy and Rzeszutko (1978) in the Lake Michigan area, comparing precipitation in Chicago, Illinois, against that of Beaver Island in Northern Lake, Michigan.

Strachan and Huneault (1979), working in the Province of Ontario adjacent to the Great Lakes found 86 percent of 50 rain samples to contain an average of 21 ng/l PCB, with an observed maximum of 120 ng/l PCB. These workers found an average of 26 ng/l in 14 rainfall samples adjacent to Lake Superior and a mean of 38 ng/l in 4 samples of snow from the Lake Superior area. In their later summary of airborne contaminants to the Great Lakes ecosystem, Eisenreich et al. (1981) report a range of 10 to 100 ng/l PCB in precipitation over the Great Lakes with an average content of 30 ng/l. These workers project total annual PCB deposition of airborne trace organic compounds to Lake Superior at 9.8 metric tons per annum (MTA), 6.9 MTA for Lake Michigan, 7.2 MTA for Lake Huron, 3.1 MTA for Lake Erie, and 2.3 MTA for Lake Ontario.

All of these studies suggested continuing inputs of PCBs to the Great Lakes ecosystem via the atmosphere, even though usage of these materials in North America was drastically curtailed in the early 1970s. For this reason, it was thought that sampling of the remote Siskiwit Lake site should be continued because the only source of contaminants to this system is apparently the atmosphere. Lake trout from Siskiwit Lake were selected for this study because of their well known predisposition to accumulate contaminants and because these fish occur both in Siskiwit Lake and in the upper Great Lakes, thus enabling direct comparison. Further, continued use of this species would permit extension of previous data from the lake and reflect changes in levels of contamination with time.

In the 1976 data set (Swain, 1978), a single composite of two lake trout was reported. These fish averaged 54.5 cm in body length, weighed an average of 1.6 kg, and contained 3.5 percent lipid. Two additional unreported composites of two lake trout each--1.5 kg and 1.7 kg average weight, and 3.2 and 3.8 percent lipid, respectively--contained 0.9 and 1.3 mg/kg PCB on a wet weight basis.

For comparison with these data, 18 lake trout were taken in 1980 and assigned to five composites as indicated in Table 1. These composites were forwarded to three participating laboratories with a request to run standard packed column gas chromatography analysis for PCBs with methods analogous and comparable with those used in the 1976 study. Laboratory 1 reported individual values for each of the five composites. These values were 3.26, 2.27, 1.57, 1.22, and 2.17 mg/kg PCB on a wet weight basis for composites 1 through 5, respectively. The reported mean value was 2.1 mg/kg PCB. Laboratories 2 and 3 homogenized the five individual composite samples and performed the analysis on a single grand composite of 18 fish. Laboratory 2 reported an observed level of 3.85 mg/kg PCB, and Laboratory 3 reported 4.3 mg/kg PCB. This yielded an average value of 3.42 mg/kg PCB for the analysis of the three laboratories. Although there was a rather large range between the three laboratories, both the individual values reported and the mean of the analyses were substantially higher than the average of 1.13 mg/kg observed in 1976.

Because of the individual variation among laboratories and because of the availability of newer, more sensitive methodologies, a blind duplicate

TABLE 1. CHARACTERISTICS OF LAKE TROUT FROM SISKIWIT LAKE, ISLE ROYALE (LAKE SUPERIOR), 1980

Composite sample	Fish per composite	Length (cm) Min.	Length (cm) Max.	Length (cm) Avg.	Avg. weight (kg)	Percent lipid
1	1	71.0	71.0	71.0	4.682	10.41
2	4	54.0	58.0	55.8	1.784	9.82
3	4	54.0	58.0	55.0	1.598	5.85
4	5	35.0	53.0	46.2	1.045	9.06
5	4	52.0	57.0	53.2	1.412	7.20
Sample mean	3.6	53.2	59.4	56.24	2.104	8.486

sample was submitted to each of the three laboratories with a request for analysis of the identical material using high resolution capillary column gas chromatography. The results, shown in Table 2, were surprising. Not only were the analyses in excellent agreement, but the mean of the analyses was well below the levels observed on Siskiwit Lake in 1976, and nearly five times lower than the packed column analyses.

To verify these results, the five Siskiwit Lake lake trout were reanalyzed as individual composites, and in addition, two fillets of lake trout were included from outer Saginaw Bay in Lake Huron. A 1.16-kg fillet was submitted taken from a 62-cm male lake trout containing 13.7 percent lipid, and the second was a 1.9-kg fillet containing 12.5 percent lipid taken from a 75-cm female lake trout. The results of the comparative analyses are shown in Table 3. In all cases, the values determined by packed column chromatography exceeded those observed with high resolution capillary column chromatography by an average of more than three times.

TABLE 2. ANALYSIS OF PCB IN SISKIWIT LAKE COMPOSITE LAKE TROUT SAMPLES BY PACKED COLUMN AS COMPARED WITH HIGH RESOLUTION CAPILLARY COLUMN GAS CHROMATOGRAPHY

Laboratory identification	Sample means total PCB packed column (mg/kg)	Sample means total PCB capillary column (mg/kg)
1	2.10	0.70
2	3.85	0.72
3	4.30	0.70

TABLE 3. ANALYSIS OF PCB BY PACKED COLUMN AS COMPARED WITH HIGH RESOLUTION CAPILLARY COLUMN GAS CHROMATOGRAPHY

Sample	Sample means total PCB packed column (mg/kg)	Sample means total PCB capillary column (mg/kg)
Lake trout fillet, male Saginaw Bay-Lake Huron	11.08	5.70
Lake trout fillet, female Saginaw Bay-Lake Huron	5.16	2.30
Lake trout-Siskiwit Lake composite number 1	3.26	1.35
Lake trout-Siskiwit Lake composite number 2	2.27	0.91
Lake trout-Siskiwit Lake composite number 3	1.57	0.57
Lake trout-Siskiwit Lake composite number 4	1.22	0.35
Lake trout-Siskiwit Lake composite number 5	2.17	0.41* 0.39*
Sample means	3.82	1.5

*Duplicate analysis.

To answer the obvious question of the reason for the differences, an intense study of the individual chromatograms was made. It quickly became apparent that a number of non-PCB peaks co-eluted in the same portion of the gas chromatographic spectrum as the PCB congeners. Careful resolution and separation with capillary column chromatography and comparison with known standards revealed that these additional peaks were derived from chlorinated turpine/camphene products characteristic of technical Toxaphene. Although, as noted previously, it was not possible to match all peaks with pure standards of Toxaphene because of biologic "weathering" of environmental samples, a consistent match of more than 20 peaks was normally achieved.

Aliquots of the same samples reported in Table 3 were reanalyzed against Toxaphene reference standards. The results of this effort are reported in Table 4. In all cases, the absolute quantities of Toxaphene-like materials exceeded the levels of PCBs observed by a considerable margin; an average difference of more than 5-fold was seen. A relatively consistent ratio of Toxaphene-like compounds to PCB congeners was observed. Only in one case, composite number 4, did this ratio become excessively large. In this case, the ratio observed was nearly double the mean of the other samples (10.6 as compared with 5.42).

For comparison with the values observed in lake trout, two water samples were taken at 1 meter of depth at the site on Siskiwit Lake indicated in Figure 2. Two additional samples were taken in Lake Superior form the 50-meter strata of water approximately 5 km southwest of Menagerie Light on Siskiwit Bay of

TABLE 4. PCB AND TOXAPHENE-LIKE COMPOUNDS (TOX) OBSERVED IN LAKE TROUT

Sample location	Total PCB (mg/kg)	Number of peaks utilized for PCB	Total TOX (mg/kg)	Number of peaks utilized for TOX	Ratio TOX:PCB
Fillet, Saginaw Bay Lake Huron	5.7	26	25.84	28	4.53
Fillet, Saginaw Bay Lake Huron	2.3	26	12.05	29	5.24
Siskiwit Lake composite no. 1	1.35	25	6.37	31	4.72
Siskiwit Lake composite no. 2	0.91	25	5.14	28	5.65
Siskiwit Lake composite no. 3	0.57	25	3.01	39	5.28
Siskiwit Lake composite no. 4	0.35	25	3.71	27	10.6
Siskiwit Lake composite no. 5	0.41* 0.39*	25 25	2.57 2.45	30 30	6.27 6.28
Sample means	1.49	25.25	7.64	29	6.07

*Duplicate analysis.

Isle Royale. The means of these samples are compared in Table 5 with the average values of samples collected in Lake Huron by staff of the Cranbrook Institute of Science, and jointly analyzed by this group and the second author. Again, a consistent ratio of Toxaphene-like material to PCB congeners was observed for all samples except those of Siskiwit Lake. Apparently because of its relative size, state of oligotrophy, and the relatively higher inputs of PCBs received, the ratio in this lake was reduced to 1.72, as compared with an average of 3.45 for the other analyses.

To assess the quantity of PCB compounds in atmospheric precipitation, a series of bulk collectors and event samplers had been set out along the Lake Huron shoreline at Hoeft State Park, Michigan, Sturgeon Point, Michigan, and Tawas State Park, Michigan. Additionally, one confirmatory station was established on the Canadian shoreline of Lake Huron below the Bruce Peninsula, and one station was established below Lake Huron at Grosse Ile, Michigan. When the results of the studies of lake water and lake trout became available, the precipitation samples were also analyzed for PCB and Toxaphene-like compounds.

The results of these analyses are shown in Table 6. Again in every case, the values observed for the Toxaphene-like substances exceeded those observed for PCBs. The mean of the ratios observed for all bulk precipitation samples, by far the majority of the data, was 3.24, which compares well with the mean ratio found for open Lake Huron water, 3.51, and with that of Lake Superior, 3.16. As was previously noted, the data points that are derived from peaks of Toxaphene-like substances of less than 20 in number should be regarded with caution. Although the data have been included for continuity, four such points exist in the data set--one from Hoeft State Park, two from Sturgeon Point, and one from the Tawas State Park sampling station. At best, the values for the Toxaphene-like substances associated with these samples should be regarded as a qualitative estimation of the amount of these substances present.

Although these data are currently being more completely analyzed, it is apparent that Toxaphene-like components make up a currently significant portion of the contaminant burden of Upper Great Lakes water, fish, and precipitation inputs. It may even be necessary to reconsider some of the historic data on PCBs in the Great Lakes in light of this new information to evaluate the possible positive interference of Toxaphene-like compounds in the analysis of these samples.

In keeping with the original purpose of this sequence of studies, a comparison of several other 1980 pesticide levels in lake trout from Siskiwit Lake also was made with the earlier data. The results of the associated analyses are shown in Table 7. In all cases for which comparative data were available, significant reductions in levels of pesticides were observed. Compounds showing such declines included the alpha isomer of benzenehexachloride (BHC, also known as hexachlorocyclohexane), heptachlor epoxide, p,p'-DDE, and p-p'-DDD. A group of new materials also was observed among which were the gamma isomer of BHC (also known as hexachlorocyclohexane, or Lindane), Toxaphene-like compounds, and the family of substances related to chlordane, including oxychlordane, *trans*-chlordane, *cis*-chlordane, and *cis*-nonachlor.

Finally, using a 1-kg homogenate of the 18 lake trout comprising composites 1 through 5 of Tables 1 through 4, negative ionization mass spectroscopy analyses were made for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The results of these analyses are shown in Table 8.

TABLE 5. MEAN VALUES OF ANALYSES OF PCB AND TOXAPHENE-LIKE COMPOUNDS (TOX) IN SELECTED WATER SAMPLES

Location	Total PCB (ng/l)	Total TOX (ng/l)	Numbers of peaks in TOX analysis	Ratio PCB: TOX
Lake Huron				
Southern Lake Huron	0.39	1.50	42	3.85
Middle Lake Huron	0.46	2.14	39	4.65
Northern Lake Huron	0.28	1.23	35	4.39
North Channel	0.77	1.60	25	2.08
Georgian Bay	0.57	1.48	36	2.60
Lake Superior Adjacent to Isle Royale	0.31	0.98	38	3.16
Siskiwit Lake, Isle Royale	1.28	2.2	37	1.72

TABLE 6. PCB AND TOXAPHENE-LIKE COMPOUNDS (TOX) OBSERVED IN SAMPLES-EVENT AND BULK PRECIPITATION, LAKE HURON

Location	Month sample taken	Sample volume (l)	Total PCB (ng/l)	Number of peaks in PCB analysis	Total TOX (ng/l)	Number of peaks in TOX analysis	Ratio TOX:PCB
Hoeft State Park sampling station	1	12.65	7.7	28	36.3	15	4.73
	2	5.86	11.1	60	21.0	31	1.88
	6	11.76	19.9	40	55.9	26	2.80
	8	15.25	1.8	40	14.5	38	8.13
	9	36.05	2.7	60	5.0	31	1.89
	10	9.85	22.9	49	51.4	32	2.24
	12	12.10	108.0	71	112.0	26	1.03
Sturgeon Point sampling station	1	5.53	87.0	65	99.0	22	1.14
	2	7.83	6.9	54	16.7	25	2.42
	5	31.05	4.3	40	17.7	23	4.11
	6	30.20	7.3	47	13.1	19	1.79
	6*	5.65	8.6	59	17.8	38	2.07
	7	30.44	1.4	36	5.58	33	3.96
	8	30.91	2.2	37	14.6	34	6.59
	8*	5.79	21.0	42	37.0	41	1.75
	9	36.75	7.6	40	16.3	34	2.15
	10	9.00	19.9	54	51.5	15	2.59
	10*	1.65	31.6	65	40.4	29	1.28
	11	18.30	9.2	41	21.3	28	2.32
Canadian shore station	8	45.72	2.6	35	26.7	22	10.38
Grosse Ile sampling station	6	28.36	5.0	43	16.2	39	3.26
	8	83.73	8.1	56	18.9	39	2.35
	10	10.44	22.9	46	74.2	32	3.24
Tawas State Park sampling station	6	24.72	8.5	42	15.6	37	1.84
	8	24.29	3.5	42	19.9	49	5.73
	9	42.15	5.3	47	11.7	36	2.21
	10	12.26	30.0	51	52.1	17	1.74
	11	24.24	4.9	38	9.7	30	1.99
	12	7.40	46.0	62	80.0	21	1.74
Mean of all samples		21.39	17.9	47.9	33.5	29.7	3.08
Mean of bulk precip. samp.		23.36	17.5	47.1	33.7	29.0	3.24
Mean of event precip. samp.		4.36	20.4	55.3	31.73	36.0	1.7

*Event samples.

TABLE 7. COMPARISON OF RESIDUE-FORMING ORGANIC SUBSTANCES OBSERVED IN LAKE TROUT (EVISCERATED WHOLE FISH) FROM SISKIWIT LAKE, 1974-1976 and 1980

Compound	Concentration (ng/g, ppb)	
	1974-76	1980
αBHC	11.0	3.5
γBHC	-	1.5
HCB	5.0	-
Heptachlor epoxide	8.0	5.2
Oxychlordane	-	5.3
trans-chlordane	-	8.8
cis-chlordane	-	24.4
p,p'-DDE	2370.0	318.0
p,p'-DDD	36.0	25.0
cis-Nonachlor	-	25.0
Toxaphene-like compounds	-	3200.0

Trace amounts of the 7 and 8 chlorine substituted PCDDs were found, but the more toxic 4 chlorine substituted isomers were not observed, nor were the 5 and 6 chlorine substituted groups. Four chlorine substituted PCDFs made up two-thirds of the total PCDFs found, the remaining one-third consisting of the 5 chlorine substituted compounds. The concentration ratio (PCDFs/PCBs) x 10^6 yields a value of 21.4 when the high resolution capillary column PCB values are used. This ratio may be of particular significance when potential impacts of PCBs on human health are considered.

CONCLUSION

The occurrence and distribution of selected persistent organochlorine compounds has been studied in the Siskiwit Lake, Lake Superior, and Lake Huron ecosystems. Although the origins of the more ubiquitous PCB compounds are not clear, historic use patterns of the pesticide Toxaphene suggest major mechanisms for long range transport of derivatives of this material to the Great Lakes. Traditionally, the vast majority of Toxaphene compounds have been used in the deep South of the United States as an agricultural insecticide against pests of cotton crops, specifically the cotton boll weevil *Anthonomus grandis* (Coleoptera: Curculioninae). Utilization patterns of Toxaphene in other areas closer to the Great Lakes suggest that only in recent years has application of this material been made as a herbicide, or as an insecticide in conjunction with the cultivation of sunflower crops in the mid-western states. Toxaphene has never been registered or approved for use in Canada. Because existing data indicate Toxaphene-like components in fish of the upper Great Lakes as early as 1974 (Swain and Glass, 1984), and because a loading rate of more than a metric ton per year would be required to observe the burdens of these Toxaphene-like materials in each of the Upper Great Lakes, it is highly likely that the majority of these components are derived from extremely long range transport (thousands of kilometers) by way of the atmosphere from areas of major utilization in the southern United States. Recent research (Rice et al., 1984), utilizing air and precipitation samples, and meteorological data, reached the same conclusion, i.e., that the probable source of the Toxaphene-like compounds observed is from the central-southern United States.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the men and women of Isle Royale Division of the National Park Service, U.S. Department of the Interior, for their continued cooperation and assistance over the several years of the project period. The cooperative analytical assistance of Drs. Jim Petty, David Stallings, Mike Ribick and Dick Schoettger of the Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, and Dr. Eliott Smith of the Cranbrook Institute of Science is acknowledged with gratitude.

The contributions of A.J."Rusty" Davis to the sampling program are remembered with appreciation.

Ook aan Carolien Schamhardt, secretaresse Vakgroep Aquatische Oecologie, Universiteit van Amsterdam, die het manuscript heeft geprapareerd, hartelijk bedankt.

TABLE 8. NEGATIVE IONIZATION MASS SPECTROSCOPY ANALYSIS OF EVISCERATED WHOLE LAKE TROUT FROM SISKIWIT LAKE (1 kg composite of 18 fish taken in 1980)

Compound	Concentration (ng/kg, ppt) by number of chlorine substitutions					
	4	5	6	7	8	Total
Chlorinated dibenzo-p-dioxins (Detection limit)	ND (1.0)	ND (1.0)	ND (2.0)	Tr. (2.0)	Tr. (3.0)	Trace
Chlorinated dibenzofurans (Detection limit)	10 (0.5)	5 (0.5)	ND (0.5)	ND (1.0)	Tr. (1.0)	15

Ratio of four chlorine PCDFs to total 10:15 = 0.67

Concentration ratio (PCDFs/PCBs) X 10^6

Packed column PCBs - (15/4.3) X 10^6 = 3.5

High resolution capillary column PCBs - (15/0.7) X 10^6 = 21.4

REFERENCES

Eisenreich, S.J., B.B. Looney, and J.D. Thornton. 1981. Airborne organic contaminants in the Great Lakes ecosystem. Environmental Science and Technology 15:30-38.

Huber, T.J. 1975. The geologic story of Isle Royale National Park. Geological Survey Bulletin 1309:1-66.

Murphy, T.J. 1976. Polychlorobiphenyls in the Atmosphere and in Precipitation in the Lake Michigan Basin. Interim Report to the U.S. Environmental Protection Agency, March 17, 1976. U.S. Environmental Protection Agency, Grosse Ile, Michigan. pp. 1-4 (Typescript).

Murphy, T.J., and C.P. Rzeszutko. 1977. Precipitation inputs of PCBs to Lake Michigan. J. Great Lakes Res. 3:305-312.

Rice, C.P., P.J. Samson, and G. Noguchi. 1984. Atmospheric Transport of Toxaphene to Lake Michigan. Report to the U.S. Environmental Protection Agency, February 1984. U.S. Environmental Protection Agency, Grosse Ile, Michigan.

Strachan, W.M.J. and H. Huneault. 1979. Polychlorinated biphenyls and organochlorine pesticides in Great Lakes precipitation. J. Great Lakes Res. 5: 61-68.

Swain, W.R. 1978. Chlorinated organic residues in fish, water, and precipitation form the vicinity of Isle Royale, Lake Superior. J. Great Lakes Res. 4:398-407.

Swain, W.R., and G.E. Glass. 1984. Potential for Pollutant Alteration of the Hydrocycle. International Association for Great Lakes Research. Abstracts of the 27th Conference on Great Lakes Research. pp. 69.

Veith, G.D., D.W. Khehl, F.A. Puglisi, G.E. Glass, and J.G. Eaton. 1977. Residues of PCBs and DDT in the western Lake Superior ecosystem. Archives of Environmental Contamination and Toxicology. 5:487-499.

BIOLOGICAL TESTING OF INDUSTRIAL EFFLUENT

by
A.M. Beym[1]

ABSTRACT

Examined are procedures for biotesting of industrial effluents. These procedures include toxicological criteria, field observations, biochemical diagnosis, and toxicogenetic research.

INTRODUCTION

Planning and organizational measures, as well as sizable capital investments, for environmental protection that have been implemented in recent years in the Soviet Union have appreciably slowed pollution of inland and continental bodies of water while significant growth has occurred in industrial and agricultural output (Federov 1977). However, development of industry, increasing industrialization, and use of chemicals in agriculture still lead to movement into the environment of a certain amount of chemicals, including xenobiotics.

The paper and pulp industry still uses much water. A trend in all countries in the 1950s has carried through to a full transition from the sulfite method to the more progressive and ecologically beneficial sulfate method of recovering pulp.

Use of new technological procedures permits maximum utilization of useful timber products for the national economy. In addition to the main product, cellulose, modern enterprises are recovering turpentine and other terpenes, tall oil, methanol, hydrogen polysulfide odorizer, furfurol, feed yeast and other products. All these engineering developments have made it possible to prevent passage of excessive carbohydrates (pentoses and hexoses), terpene hydrocarbons, resin and fatty acids, alcohols, sulfur-containing agents of the methylmercaptan class, and other chemical compounds into liquid sewage.

---

[1] Institute of Ecological Toxicology.

Treatment plants serve as barriers to prevent pollution of water by sewage. Within a short period of time, 85% of the enterprises of the paper and pulp industry were supplied with biological treatment installations. Two combines, the Baykal Paper and Pulp Combine (BTsBK) and the Selenginsk Pulp and Cardboard Combine, have integrated purification processes consisting of three steps: biological, chemical, and mechanical. Other plants are equipped with mechanical purification methods and systems of aerating ponds. The treatment installations that have been started up recently were designed on the basis of progressive water-consumption standards and existence of an efficient intraplant system of purifying liquid sewage.

Work dealing with environmental protection and optimum use of natural resources is acquiring increasing importance with each passing year. In the USSR, sanitary-hygienic, water-management, and other environmental protection requirements imposed on construction and operation of industrial enterprises are growing increasingly strict. This requires additional work on problems of environmental protection at all organizational stages: scientific research, designing and building enterprises, and routine industrial work (Tipisev 1984).

Liquid effluent from different types of plants contain diverse chemical compounds, the number of which may exceed several tens or hundreds. It is possible to identify the chemical composition of this multicomponent flow, but it is unrealistic to implement thorough and regular monitoring of these substances. For this reason, inspection of quality of industrial sewage is effected in our country and abroad mainly by performing hydrochemical analyses of basic overall indicators and so-called priority pollutants. For example, the following mandatory parameters for hydrochemical monitoring have been defined for treated liquid sewage of the Baykal and Selenginsk combines: active medium reaction (pH), temperature, color index, biological oxygen demand (BOD_5 and BOD_{full}), chemical oxygen minimum (COM), total mineralization, volatile phenols, and total organic sulfides. In a number of cases, when treated sewage is discharged into reservoirs of a special category, which are used for fisheries and have recreational value, additional parameters are monitored: levels of iron and turpentine, methanol, volatile fatty acids, carbohydrates, and others. In spite of expansion of the list of monitored parameters, however, it is not feasible to efficiently determine the quantities of the entire range of chemical compounds, which makes it difficult to provide a biological assessment of the quality of sewage dumped into bodies of water.

BIOLOGICAL TESTING OF INDUSTRIAL SEWAGE

In recent times, methods of direct evaluation of toxicity of the water environment are gaining increasing importance, i.e., biotesting of water quality by means of sensitive hydrobionts. Biotesting amounts primarily to checking toxicity of industrial waste. In addition, it is equally important to use biotests for determination of toxicity of polluted natural waters, and this is already a matter of biological monitoring of their quality. In particular, this makes it possible to use biotesting in the system of monitoring the quality of natural waters, as is being done in our country and abroad (Braginskiy 1978, Izrael' et al. 1978).

Biotesting opens up vast opportunities for monitoring water quality on a quantitative basis, since it yields concrete figures characterizing toxicity of the water environment for hydrobionts. The results of biotesting are of considerable interest, not only in the ecological, but hygienic aspects. On the one hand, biotesting is used in hygienic studies as a rapid method of assessing toxicity of the water environment and determining the nature of change in it under the influence of different factors (Krasovskiy et al. 1983). On the other hand, hydrobionts are representatives of a complicated aquatic biocenosis, which is actively involved in processes of spontaneous self-purification of water to remove pollutants. Consequently, the toxic effect of chemicals on them could lead to decline of self-purifying capacity of a reservoir and worsening of its sanitary conditions, which is already of interest from the purely sanitary and hygienic point of view (Beym et al. 1984).

TOXICOLOGICAL CRITERIA FOR ASSESSING QUALITY OF TREATED LIQUID SEWAGE

Studies dealing with biotesting of sewage, in particular the industrial waste from the BTsBK (Izrael' et al. 1981), hold an important place in the set of state measures to protect the flora and fauna of Lake Baykal against anthropogenic pollution. Biological studies of liquid waste preceded development of a basic industrial plan for integrated purification of industrial waste at the BTsBK [Baykal Paper and Pulp Combine]. On the basis of model and semiproduction experiments, marine toxicologists demonstrated that introduction of methods of biological and chemical treatment of liquid waste leads to significant decline of water pollution in reservoirs.

From the very first days of operation of the BTsBK (1967), a biological service for monitoring liquid waste quality was organized. The purpose of the studies included:

- Multilevel investigation of toxicity of untreated and treated liquid sewage from sulfate and cellulose plants to aquatic organisms.

- Biological assessment of qualitative composition of liquid sewage.

- Determination of vital (inactive) concentrations of a number of organic and inorganic substances contained in runoff.

Typical representatives of plankton and benthos (algae, protozoans, helminths, mollusks, crustaceans), as well as fish at different stages of development (roe, larvae, young and adult specimens) were the objects of marine toxicological studies. The distinction of the toxicological work done in Lake Baykal was the difficulty of choice of test objects, since the lake's ecosystem is unique with respect to presence of endemic species that play the leading role in processes of transforming matter into energy.

The following served as indicators of the reaction of test organisms to presence in water of toxic impurities: survival rate, poisoning symptoms, changes in reproduction and fertility, a set of pathophysiological and biochemical changes, and others. The vital properties of liquid waste were evaluated in acute (24, 48 and 96 h), subacute (30 days) and chronic (6-9 months) experiments. The results of scientific research pursued for more than 15 years revealed that, after combined purification, liquid waste has mild toxicity (even if not diluted) for most endemic and palearctic aquatic organisms. The degree of toxicity of industrial waste to hydrobionts diminishes substantially if it is diluted unadulterated water (gradually reaching zero toxicity) (Table 1).

TABLE 1. RESULTS OF BIOTESTING TREATED LIQUID WASTE FROM THE BTsBK (1967-1984)

Test Objects	Vital Degree of Dilution
Algae	1:1 - 1:10
Chlorella pyrenoidosa	
Chara sp.	
Scenedesmus quadricauda	
Protozoa	1:1
Paramaecium caudatum	
Turbellaria	1:4 - 1:10
Baicalobia guttata	
Annelides	1:1 - 1:4
Oligohaeta	
Tubifex tubifex	
Lumbriculus variegatus	
Lamprodrilus nigrescens	
Mollusca	1:2 - 1:5
Benedictia baicalensis	
Crustacea	1:1 - 1:10
Cladocera	
Daphnia magna	
D. pulex	
D. longispina	
Chydorus sphaericus	
Symocephalus vetulus	
Copepoda	
Cyclops kolensis	
Epischura baicalensis	1 : 50

TABLE 1 (continued)

Test Objects	Vital Degree of Dilution
Amphipoda	1:1 - 1:8
Gammarus lacustris	
Eulimnogammarus verrucosus	
Eulimnogammarus cyaneus	
Acanthogammarus victorii	
Pisces	1:6 - 1:20
Coregonus autumnalis migratorius	
Thymallus arcticus baicalensis	
Paracottus kessleri	
P. kneri	
Cottocomephorus grewingki	
Rutilus rutilus lacustris	
Lenciscus lenciscus	
Phoxinus phoxinus	

Special tracer studies using the gold isotope Au-198 revealed that treated liquid waste is diluted by a deep-lying dispersing device to 1/20th or more at the dumping site and to more than 1/100 at a distance of 500 m or more (Vetrov and Dekin, 1977). These data indicate that diluted sewage in water that is being mixed does not have a devastating effect on representatives of the aquatic biocenosis, including such particularly sensitive species as the endemic little crayfish, Epischura baicalensis Sars.

FIELD STUDIES AND OBSERVATIONS: ICHTHYOLOGICAL MAPPING

In all periods of observation, fishing-crib experiments were performed with representatives of the ichthyofauna (Baykal cisco--Coregonus autumnalis migratorius Gorgi, grayling--Thymallus arcticus baicalensis Dybowski, bullhead--Cottus Knei Dybowski, Cottus Kessleri Dybowski, minnow--Phoxinus phoxinus Linne) in the immediate zones of dilution of industrial waste and along the cone of their distribution in a north-easterly direction along the shoreline. The fish in the cribs, which were placed at different depths, survived in these zones without being fed for 48 days or more. Control catches in this region with special fishing gear revealed presence of diverse representatives of ichthyofauna of Lake Baykal. Organoleptic studies showed that the fish had no odor whatsoever. Concurrently, cytologists, geneticists, and physiologists investigated cytogenetic changes in the cells of some fish tissues, behavioral reactions of fish and gammarids exposed to low doses of liquid waste. Electro-physiological monitoring methods were used to observe changes in the chemo-receptor system of fish and crustaceans, as well as biochemical criteria for evaluating stress reactions of fish and the basic parameters of carbohydrate, fat and protein metabolism.

BIOCHEMICAL EVALUATION OF FUNCTIONAL STATE OF FISH IN BIOTESTING OF INDUSTRIAL LIQUID WASTE

One of the most important indicators that determine the resistance of living systems to adverse (toxic) factors is their reactivity.

In biology, reactivity usually refers to the organism's capacity for an adequate reaction in response to altered endogenous and exogenous conditions. In other words, the highest levels of reactivity enable a living system to respond in the most advantageous way to diverse factors and preserve homeostatic functional parameters of vital systems.

It should be emphasized in particular that optimum forms of adaptation to adverse factors do not necessarily provide for maintaining stability of the organism's endogenous environment as a whole. Moreover, optimum level of reactivity also refers to the capacity of higher integrative regulatory elements of the organism to select the particular systems (which differ when exposed to essentially different factors), the activity of which must be in homeostasis. Deviations in their functional parameters must not exceed the physiological range. At the same time, the functional activity of other systems can change over the widest range without detriment to the organism as a whole.

In the light of these conceptions, when conducting toxicological studies of water, it is necessary to make a more distinct differentiation between the results of observations of so-called target systems, i.e., those to which the effect of a given toxicant is addressed, and purely regulatory changes, which are necessary to compensate for functional deviations in organs or target systems.

When performing biotests on fish, we made an attempt at biochemical determination of the functional state of systems both directly related to detoxification of compounds contained in waste from the BTsBK and reactions of the organism that are instrumental in maintaining optimum levels of reactivity.

In this regard, it is of special interest to investigate the system of enzymes that catalyze oxidative metabolism of hydrophobic compounds (Kozlov et al. 1983a, 1983b), localized in membranes of the liver's endoplasmic reticulum. This system, which consists of two membrane proteins of NADP·H cytochrome P-450 reductase and cytochrome P-450, is capable of utilizing virtually any hydrophobic compounds in toxic agents. Moreover, these oxygenases participate in the metabolism of steroid hormones and fatty acids. An interesting distinction of this oxygenase system is that a number of organic compounds (barbiturates, polycyclic hydrocarbons, etc.) are capable of inducing synthesis of new molecules of cytochrome P-450 in hepatic microsomes.

As a result of the experiments, the following results were obtained for concentrations and activity of microsomal oxygenases (Table 2). Lake Baykal is one of the cleanest bodies of water, and for this reason the demonstrated low concentrations of cytochrome P-450 in the liver of local fish is due to the exceptionally low levels of NADP inductors, on the one hand, and on the other hand it enables us to consider the demonstrated level of development of systems of oxygenases with mixed function to be constitutive, used primarily for metabolism of endogenous substrates with synthesis of steroid hormones and bile acids.

This hypothesis is also confirmed by the fact that we demonstrated exceptionally low activity of benz-α-pyrene hydroxylase, which does not exceed 0.5 pmol phenol metabolic products of benz-α-pyrene/mg protein/min, which is 1/50th the activity inherent in warm-blooded animals and 1/10th the activity of the liver of some salt-water fish.

TABLE 2. LEVELS AND ACTIVITY OF CYTOCHROME P450 IN LIVER OF LAKE BAYKAL FISH

Fish	Cytochrome P-450, nmol/mg microsomal protein	Activity of NADP·H cytochrome C reductase, nmol cytochrome C mg/min
Phoxinus Phoxinus Phoxinus (Linne)	0.8 ± 0.13	2.49 ± 0.99
Perca fluviatilis1(Linne)	0.11 ± 0.06	0.60 ± 0.20
Rutilus rutilus (Linne)	0.31 ± 0.12	1.17 ± 0.31
Thymallus arcticus baicalensis (Dybowski)	0.04 ± 0.02	0.61 ± 0.01
Coregonus autumnalis migratorius (Georgi)	0.04 ± 0.01	0.58 ± 0.07
Procottus jeitelesi	0.21 ± 0.05	-

It was of special interest to investigate the effect of waste from paper and pulp plants on the system of microsomal oxygenases of fish in Lake Baykal. For these experiments, fish (perch, grayling and cisco) caught both in the immediate vicinity of dumping from the BTsBK and other parts of Lake Baykal were adapted to living in tanks with Baykal water for 5-7 days. After this, the adapted fish were exposed to treated sewage in a concentration of 1:20. We analyzed levels of cytochrome P-450, activity of NADP·H cytochrome P-450 reductase, as well as kinetics of enzymatic peroxidation of lipids induced by NADP·H cytochrome P-450 reductase and cytochrome P-450, in microsomal fractions of the liver of these fish. The levels of cytochrome P-450 were the same in microsomal fractions from the liver of fish exposed to treated sewage and in the liver of control fish. This indicates that, under these experimental conditions, the liquid sewage apparently contained no polycylic hydrocarbons in concentrations sufficient for induction of microsomal oxy-reductases. This also is confirmed by the fact that neither the kinetics of cytochrome C reduction nor rate of lipid peroxidation in microsomal fractions of the liver of fish kept in the run-off from the BTsBK in a dilution of 1:20 differed from control values (Kotelevtsev et al. 1984).

It also is known that mechanisms of nonspecific adaptation are well-represented in cold-blooded animals. The system of hormonal regulation of physiological functions and hemoglobin system are the most labile in reactions to changes in environmental conditions. The nature of physiological changes in response to chemical stress indicates that the general adaptation syndrome develops in fish (Luk'yanenko 1967). There are no clearcut and validated explanations, however, for the mechanisms of this phenomenon due to lack of studies of different aspects of hormonal physiology of fish. This makes it difficult to furnish a biochemical diagnosis of functional state of fish under stress in aquatic toxicological studies. The role of adrenocortical hormones in regulation of systemic adaptive reactions is known. The data on glucocorticoid levels in fish blood are contradictory, and this is attributable to the flaws in analytical methods (Wedemeyer et al.1981).

The radioimmunological method we use permits very accurate evaluation of the system involved in nonspecific resistance and choice of optimum forms and levels of behavior in teleost fish under normal conditions and with exposure to chemical anthropogenic factors. In control experiments, corticosterone was demonstrated in blood plasma of examined fish in the following concentrations, ng/ml: grayling--3.3±0.4 (n = 16), cicso--6.2±0.5 (n = 37), perch--5.5±0.8 (n = 4) and burbot--2.1±0.3 (n = 3). A preliminary series of studies failed to demonstrate sex-related differences in levels of this hormone. The condition of the system that provides for nonspecific resistance was studied with exposure to the set of chemical factors contained in treated liquid waste from sulfate-cellulose plants (BTsBK). Corticosterone level constituted 2.4±0.3 ng/ml (n = 14) in experimental grayling, and it did not differ with statistical reliability from normal physiological values.

At the present time, it has become possible to develop special systems adapted for selective detection of steroid compounds in fish with mineralocorticoid and glucocorticoid activity. Assays were made of concentrations of aldosterone, 11-deoxycorticosterone and cortisol in samples of blood from intact fish--grayling and cisco. It was demonstrated, in principle, that factors differing in nature and duration of exposure (impairment of abiotic environmental factors) elicit changes in relative concentrations of glucocorticoid and mineralocorticoid hormones with an adaptive effect. Thus, the radioimmunological method of assaying adrenocortical hormones permits more accurate evaluation of the system of nonspecific resistance in teleost fish under normal conditions and in the presence of chemical pathology caused by anthropogenic factors, and it can be used with success for biological testing of industrial liquid waste.

Studies of different fractions of hemoglobin (Hb) were conducted in the Baykal cisco caught in a net in the southeastern part of Lake Baykal. During the adaptation period, the fish were kept in tanks with running water from the lake (t = 8-10°C). It was ultimately found that the levels of alkali-resistant Hb differed significantly in different species of fish (ranging from 11.9% in the grayling to 25.5% in the cisco). There was similar variation in concentration of carboxyhemoglobin (from 0.86% in the grayling to 1.38% in the burbot) and methemoglobin (MetHb) (from 3.6% in the cisco to 6.7% in the perch and burbot). Methemoglobin content depends on blood levels of oxidants that change Hb iron to a trivalent state. In the course of oxidation of Hb to MetHb there is formation of oxygen superoxide anion with high reactivity.

One of the most important enzymes of antioxidant protection of the organism is superoxide dismutase (SOD), which causes dismutation of superoxide anion. High SOD activity is demonstrable in the blood of the examined fish: from 1.18 to 10.93 arbitary units per mg Hb. On the whole, analysis revealed that SOD activity is higher in cold-blooded animals than in warm-blooded.

Investigation of the effect of liquid sewage on parameters of Hb composition in perch and grayling revealed the same direction of changes in methemoglobin and carboxyhemoglobin content: increase in concentration of oxidized Hb (methemoglobin), with decrease in CO-bound Hb (carboxyhemoglobin). Evidently, there are substances in liquid waste that are instrumental in converting hemoglobin to an irreversibly oxidized form (MetHb).

As a rule, the amount of alkali-resistant Hb increases under the effect of adverse environmental factors. Under physiological conditions, higher resistance to the denaturing effect of alkali is inherent in Hb at the early developmental stages--embryonic and fetal. It was also found that diluted liquid sewage from the aerating pond of the BTsBK could not alter significantly either the Hb fractions studied or the activity of one of the enzymes of the antioxidant system of red blood cells--superoxide dismutase.

Thus, development of modern biotesting methods requires use of the results of basic research in different branches of biology, including biochemical studies on the membrane and molecular levels. Indeed, identification of the intimate mechanisms that protect functional systems of the cell against the toxic effects of poisons, pollutants and their naturally occurring metabolites permits not only development of highly sensitive and specific tests for biological monitoring of the environment, but prediction of mechanisms of subsequent effects of xenobiotics and their metabolic products on aquatic animals.

TOXICOGENETIC STUDIES

A modification of the semiquantitative Ames test--salmonella/microsomes with use of a system of metabolic activation from the liver of the Baykal cisco induced by methylcholanthrene--was developed for investigation of the toxic genetic effect of constituents of waste from the paper and pulp industry. Preparations of microsomal fractions from the cisco liver are highly active, as are fractions from the liver of induced rats.

Investigation of a series of samples of sewage from the Baykal Paper and Pulp Combine collected at different times from different stages of production and treatment using the Ames test with a system of metabolic activation from the liver of fish and rats, revealed that the composition of liquid waste was heterogeneous, as manifested by the existence of direct mutagenic activity in some of the samples taken from the first and intermediate stages of production and treatment. No genetic activity was found in virtually all samples taken from the last stage of sewage treatment (Glazer et al. 1984).

BIOTESTING OF CONSTITUENTS OF LIQUID SEWAGE

The waste from paper and pulp enterprises is characterized by specific organic substances that make up the wood, as well as those formed in the course of the technological operation for production of cellulose and treatment measures. For this reason, investigations to set the scientifically validated maximum allowable concentrations (MAC) of substances that are a potential hazard to aquatic organisms acquire importance.

Experimentation is the principal method of establishing the threshold of toxicity of chemical compounds. MAC is determined for the weakest biological element that is damaged by the lowest concentrations of pollutant and it becomes the minimum factor in assessing reservoirs. In addition to the known method of fish samples and survival of other aquatic organisms, a wide assortment of physiological, biochemical and biological tests is used as a criterion of toxicity, and together they made it possible to determine the concentrations of reagents at which the disturbances in vital functions of hydrobionts are at the stage of prepathological development. Special attention was devoted to long-term experiments to determine the chronic adverse effects of low doses of toxicants and to experiments on organisms with a short cycle of development in order to assess the genetic sequelae of poisoning.

Many years of toxicological research established the vital permissible concentrations of a number of deleterious elements in waste from the paper and pulp industry. These substances are referable to different classes of chemical compounds: aliphatic and terpene hydrocarbons, organic sulfides, aromatic hydrocarbons of the phenol class and others. Taking into consideration the toxicological indicator of deleteriousness, biologically validated MAC were set for 30 components, including such agents as carbolic acid (phenol), *ortho*-cresol, *meta*- and *para*-cresol, guaiacol and others.

For the first time in biological practice, studies were made of degree of harm to hydrobionts of suspended substances (residue of cellulose fibers and lignin).

The levels of all these substances in liquid sewage are strictly limited and monitored on the basis of chemical analyses made by laboratories of the State Committee for Hydrology and Metallurgy [Goskomgidromet].

Toxicometric analysis for hydrobionts is made not only of individual components, but different combinations (total monatomic phenols, total multiatomic phenols, combinations thereof mixed with sulfur-containing compounds of the methylmercaptan class and others). The MAC are used to assess the degree of toxicity of formed industrial liquid waste. Ultimately, these biological standards determine the extent of capital investments in planning and building treatment plants, and they assure purity of lake water.

BIOMONITORING AS A COMPONENT AND INTEGRAL PART OF BIOTESTING INDUSTRIAL WASTE

In order to solve problems of biotesting, it is important to select and develop "key" or model territories that are exposed to industrial liquid sewage. The area of scattered purified liquid waste from the Baykal Paper and Pulp Combine is one of these testing areas of permanent monitoring in South Baykal (Kozhova 1971, Kozhova et al. 1965). Biomonitoring is performed with particular thoroughness in a section that is adjacent to the site of discharge of sewage. Material is collected in sections at distances of 0.05, 0.1, 0.5 and 1 km in the dumping region. Each section consists of several stations in different depth zones, i.e., different biotopes.

A control testing area is situated outside the area affected by dumped sewage. In addition to chemical composition of the water, studies are made of both plankton and benthos communities, including water and soil bacteria. The following are determined in plankton communities: composition and quantity of phytoplankton; primary production by the oxygen and radioactive carbon methods; total quantity of bacterioplankton by the method of ultramembrane filtration; basic physiological groups of bacteria; population of bacterioplankton by the method of separate tests for total number and dark assimilation of ^{14}C; and composition, number, biomass and production of zooplankton determined by indicators of growth and reproduction. Samples are taken along the entire column of water from standard hydrological levels using a (Dzhedi) net and bathymeter. Benthos is studied with consideration of vertical zonality of communities and composition of soil at different depths, with use of scuba divers who determine the projective cover of the bottom and organisms that are difficult to detect with the usual collecting gear (Kozhova 1974).

A comparison of all the communities studied revealed that an anthropogenic effect is manifested in the zone of a small spot of polluted soil (0.1 km^2) at the lake's bottom (beyond the littoral zone), where there is accumulation of anthropogenic sediment. There, microbiological indicators, total biomass of zoobenthos and proportion of dominant animals in the biomass--gammarids, mollusks and Oligochaetae--undergo substantial change. For example, at a depth of 5-20 m, the mollusk and Oligochaetae community of such soil changes to a gammarid community. In the zone at a depth of 20-50 m, an Oligochaetae community changes into a gammarid one. The same happens in the zone at a depth of 50-100 m. Gammarid biomass undergoes the least change. However, even in gammarids, as in the other animals studied, there is a reduction in species composition.

Alteration of communities also occurs in less polluted soil, where the anthropogenic sediment is less marked. Interestingly, at some depths the macrozoobenthos on such soil is even larger than in control sections. At a depth of 5-20 m, this happens due to increased profusion of mollusks and at 20-50 m, Oligochaetae. But in the 50- to 100-m zone, zoobenthos biomass also decreases on less polluted ground. This is particularly noticeable for Oligochaetae biomass.

A comparison of the parameters used for ecological mapping enables us to rank them in the following order according to significance to water quality in Lake Baykal: bacteria at the bottom and zoobenthos > bacterioplankton > primary production > zooplankton > phytoplankton > littoral phytobenthos.

Let us note that the zone of impact of the Baykal Paper and Pulp Combine remained at virtually the same level in the course of a 10-year observation period (Izrael' et al. 1978).

Thus, biotesting of industrial sewage, unlike traditional analytical methods of monitoring or in addition to these methods, makes it possible to determine water quality on a quantitative basis and characterizes the extent of toxicity of the aquatic environment for hydrobionts. It was demonstrated with laboraory biotests that 24-h water from the sulfate-pulp industry has virtually no acute toxicity after integrated three-step purification (biological, chemical, mechanical) for most of the endemic and palearctic hydrobiont species examined. The results of chronic experiments revealed that the degree of toxicity of liquid waste for aquatic organisms diminishes when diluted in natural water (from 2- to 50-fold). Vital dilution of industrial waste in the lake is obtained over a radius of 100 m from the heads of the deep scattering dumping devices.

Modern methods of physiocochemical and molecular biology hold an important place in biotesting, with regard to diagnosing pathophysiological states. Studies have been made of mixed function monoxygenase systems of the liver of Baykal fish, which affect oxidative metabolism of xenobiotics. It was shown that the level of microsomal fraction cytochrome P-450 in fish, as well as level of induction in membranes of the hepatic endoplasmic reticulum of phospholipid peroxidation,can serve as a test to evaluate pollution of the water environment. Using spectrophotometry and disk electrophoresis, it was established that the waste from the BTsBK in a ratio of 1:20 does not affect the level of cytochrome P-450 and, unlike the tested exogenous xenobiotics (3-methylcholanthrene), does not elicit synthesis of cytochrome P-450 isoforms.

All of the results of integrated studies are used in routine work for experimental evaluation of toxicity of treated liquid waste from the Baykal Paper and Pulp Combine, and they serve as the basis for determining conditions under which waste is dumped to preclude any possible harm to the lake's biocenosis.

Biotesting problems are multifaceted, and they are far from being solved. One of the tasks for the next few years is to develop automated biotesting systems, which would facilitate considerably the work of biologisttoxicologists and yield results faster. It would be useful and desirable to specially discuss these aspects of biotesting within the framework of Soviet-American collaboration.

BIBLIOGRAPHY

Beym, A.M., G.N. Krasovskiy, I.V. Sutokskaya, and L.Ya. Vasyukovich. 1984. Hygienic-ecological approaches to biomonitoring of water quality. In: Samoochishcheniye vody i migratsiya zagryazneniy po troficheskoy tsepi (Water Self-Purification and Migration of Pollutants Over the Trophic Chain). Moscow, USSR. pp. 16-22.

Braginskiy, L.P. 1978. New system for biomonitoring the aquatic environment in the United States. Gidrobiol. Zhurn. 14(1):77-83.

Fedorov, Ye.K. 1977. Ekologicheskiy krizis i sotsial'nyy progress (The Ecological Crisis and Social Progress). Leningrad, USSR, 176 pages.

Glazer, V.M., V.M. Savov, S.K. Abilev, et al. 1984. A test system for biomonitoring based on membrane-bound enzyme complexes. 4: Evaluation of toxicogenetic effects in Ames test system with metabolic activation by microsomal monoxygenases from fish liver. Biol. Nauki, 5:85-89.

Izrael', Yu.A., N.K. Gasilina, F.Ya. Rovinskiy, and L.M. Filippova. 1978. Osushchestvleniye v SSSR sistemy monitoringa zagryazneniya prirodnoy sredy (Implemention of System for Monitoring the Environment in the USSR). Leningrad, USSR. 67 pages.

Izrael', Yu.A., Yu.A. Anokhin, B.B. Chebanenko, et al. 1981. Integrated analysis of environmental and validation of monitoring in the region of Lake Baykal. In: Vsestoronniy analiz okruzhayushchey prirodnoy sredy (trudy IV Sovetsko-amerikanskogo simpoziuma, Dzhekson, Vayoming, 22-27 oktyabrya 1979 g.) (Comprehensive Analysis of the Environment, Transactions of 4th Soviet-American Symposium, Jackson, Wyoming, 22-27 October 1979), Leningrad, USSR. pp. 43-59.

Kotelevtsev, S.V., V.M. Glazer, V.B. Ritov, et al. 1984. Methods of physicochemical biology for ecological and toxicological evaluation of waste dumped by enterprises of the paper and pulp industry. In: Problemy okhrany prirody. Tezisy dokl (Problems of Environmental Protection. Summaries of Papers). Baykalsk, USSR. pp. 86-87.

Kozhova, O.M., L.A. Izhboldina, and G.S. Kaplina. 1965. Benthos of littoral and sublittoral region along eastern shores of Lake Baykal. Gidrobiol. Zhurn. 1(4):14-21.

Kozhova, O.M. 1971. Current status of fauna and flora of Lake Baykal in the region of dumping industrial waste by Baykal plant. In: Issledovaniya gidrobiologicheskogo rezhima vodoyemov Vostochnoy Sibiri (Investigations of Hydrobiological Conditions in Waters of East Siberia). Irkutsk, USSR. pp. 3-9

Kozhova, O.M. 1974. Yearly changes in biocenoses of the Utulik-Murin region of South Baykal. In: Produktivnost' Baykala i antropogennyye izmeneniya yego prirody (Productivity of Lake Baykal and Anthropogenic Changes in It). Irkutsk, USSR. pp. 160-171.

Kozlov, Yu.P., V.Ye. Kagan, A.M. Beym, et al. 1983. Test systems for biomonitoring based on membrane-bound enzymatic complexes. 1: Investigation of oxygenases with mixed functions in liver microsomes of endemic fish of Lake Baykal. Biol. Nauki. 1:20-24.

Kozlov, Yu.P., V.Ye. Kagan, A.M. Beym, et al. 1983b. Test systems for biomonitoring based on membrane-bound enzymatic complexes. 2: Investigation of enzymatic and nonenzymatic systems of lipid peroxidation in liver microsomes of endemic fish of Lake Baykal. Biol. Nauki. 5:18-23.

Krasovskiy, G.N., I.P. Pletnikova, and I.V. Sutokskaya. 1983. Criterion of deleteriousness in evaluation of toxic effect of chemical pollution of water. In: Teoreticheskiye problemy vodnoy toksikologii (Theoretical Problems of Water Toxicology), Moscow, USSR. pp. 42-48.

Luk'yanenko, V.I. 1967. Toksikologiya ryb (Toxicology of Fish), Moscow, USSR. 216 pages.

Tipisev, A.Ya. 1984. Environmental protection--a priority task. Bumazhnaya Promyshlennost'. 5:1-3.

Vetrov, V.A. and S.A. Dekin. 1977. Investigation of distribution of impurities using radioactive tracer. In Techeniya v Baykale [Currents of Lake Baykal], Novosibirsk, USSR. pp. 133-143.

Wedemeyer, G.A., F.P. Meyer, and L. Smith. 1981. Stress and diseases of fish. Moscow, USSR. 128 pages.

COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM

by

R.C. Russo[1], A. Pilli[2], and J. Crane[2]

ABSTRACT

The Complex Effluent Toxicity Information System (CETIS) is a computerized data management system designed to assemble the results of effluent toxicity tests so that toxicity characteristics of complex effluents can be determined on an industry-by-industry basis. Data are obtained through literature searches of published reports and from individuals who provide unpublished bioassay data from their state or regional biomonitoring programs. Before entry into the computer file, the data are evaluated by reviewers who are experienced in bioassay methods and trained in CETIS procedures. In June 1984, data from 500 references for 1500 bioassay tests were in the CETIS data base.

INTRODUCTION

Toxicity data from bioassays with freshwater and saltwater organisms are used in assessing the effects of complex effluents on the aquatic environment. Such information can be used in setting pollutant discharge limits for industries and municipalities for wastewaters being discharged into natural aquatic systems, in determining where to use toxicity testing, and in interpreting toxicity test results.

Much of the toxicity data on complex effluents is available in unpublished form, although some information has been included in reports and journal articles dealing with biomonitoring or with environmental impacts of specific kinds of wastewaters. Bioassay methods used for complex effluent testing are available in the literature (U.S. EPA 1971, Peltier 1978, APHA et al. 1981, Weber and Peltier 1981, Peltier and Weber 1984).

---

[1] Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, GA 30613.

[2] Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, MN 55804

To make such data widely available to researchers and to those involved in setting water quality standards, all obtainable published and unpublished data have been compiled in the Complex Effluents Toxicity Information System (CETIS).

The CETIS data base is a computerized data management system designed to assemble the results of effluent toxicity tests so that toxicity characteristics of complex effluents can be determined on an industry-by-industry basis. The information contained in the data base is available from the U.S. Environmental Protection Agency, Environmental Research Laboratory in Duluth, Minnesota, or through the National Computer Center in Raleigh, North Carolina.

DESCRIPTION AND METHODS

Data to be entered in the data base are obtained in two major ways. First, literature searches identifying toxicity tests of complex effluents are performed by computer bibliographic retrieval. Published reports are screened for suitability for CETIS, and those containing useful information are incorporated into the system. Second, a coordination network consisting of regional contact persons throughout the United States is in operation. These individuals are involved with state or regional biomonitoring programs and provide unpublished bioassay data for inclusion in the system.

Published reports and unpublished data are evaluated by reviewers who are experienced in bioassay methods and trained in the procedures of the CETIS system. The bioassay information in a published or unpublished report or on unpublished data sheets is carefully examined by a technical reviewer to identify, extract, and record those data suitable for entry into the data base. Appropriate information is entered onto data record forms and entered into computer data files on a PDP 11/70 computer.

Information extracted from the published and unpublished data reports is grouped into 12 general categories: Facility Information, Effluent/Receiving Water Information, Reviewer Name/Coding Date Information, Sampling Information, Toxicity Test Information, Test Water Information, Other Water Profile Information, Test Organism Information, Dilution Water Information, Test Method Information, Test Result Information, and Remarks. Data encoded consist of both data elements unique to CETIS and data elements obtained through cross-reference from other National Computer Center data systems.

Information included under "Facility Information" includes the facility name (direct or indirect discharger), discharge number, pipe number, facility type, facility address, receiving water, and basin.

The types of wastewaters included in the data base are organized according to the general categories of industry type, such as: ore mining, coal mining, textile mill operations, timber products processing, pulp and paperboard mills and converted paper product operations, paint and ink formulating and printing, inorganic chemicals manufacturing, plastics and synthetic materials manufacturing, miscellaneous chemical manufacturing, organic chemical manufacturing, soap and detergent manufacturing, petroleum refining, paving and roofing material manufacturing, rubber processing, leather tanning and finishing, iron and steel manufacturing, nonferrous metals manufacturing, machinery and mechanical products manufacturing, electroplating, electric services, laundry operations, and sewerage system operations.

Discharge types are identified as to whether a wastewater is a cooling water, process water, or both; or whether it is some other type of of discharge such as storm water runoff. Receiving waters are identified as to name of water body, name of major and minor basin, and river reach. Other information regarding the receiving water is also included, if available, such as mean annual flow. Treatment processes to which a tested wastewater was subjected are also noted in the encoding process. These treatment processes are grouped into five categories: Physical Treatment (for example, ammonia stripping, floatation, multimedia filtration), Chemical Treatment (for example, carbon adsorption, chlorine disinfection, ion exchange), Biological Treatment (for example, activated sludge, nitrification-denitrification), Sludge Treatment and Disposal (for example, aerobic or anaerobic digestion), and Other Processes (for example, rueuse or recycle of treated effluent).

Sampling information on the bioassay test sample is recorded, such as collection date and time, how the sample was obtained (continuous sampling, grab sample, or composite sample), and whether the sample was an actual field sample or a spiked or synthetically prepared sample. If available, the flow from the discharge pipe at time of sampling is entered.

Identifying information is provided about the bioassay, such as test date and time, testing organization, test duration, effluent concentrations tested, exposure type, residue analysis, and bioassay type. Exposure types include static, renewal, flow-through, and diet tests. Bioassay types include screening tests, acute (short-term) tests, and partial or full life cycle tests. Effluent concentrations tested may be expressed as grams, milligrams, or micrograms per liter, or as percent effluent in the test solution. Characteristics of the test water are then entered. This includes such information as the concentration ranges for the test water, dissolved oxygen, pH, temperature, alkalinity, and hardness. The mean for each of these parameters is recorded if available. The range includes any duplications or replications of the dilution series concentrations. Test water chemical data for screening tests are reported for the 0 percent and 100 percent effluent concentrations. If test water data are not reported, diluent or effluent values may be used.

If measurements of additional water chemical characteristics have been reported by the experimenter for the bioassay test water, effluent, or diluent (such as, for example, anions, metals, nonmetal cations, organics), these are briefly identified as to their availability in the original report.

Aquatic species used in bioassays included in the CETIS are fish, macroinvertebrates, amphibians, and aquatic plants. Table 1 provides a list of test species for which toxicity data are available in the CETIS. The lifestage of test organisms is identified as one of the following: alevin, adult, egg, eyed egg, fertilized egg, fingerling, fry, instars, juvenile, larval, neonate, nymph, underyearling, young, yearling, or zoea. The ages, weights, and lengths of test organisms are recorded, and the source (cultured, field collected, hatchery, etc.) is identified. Information is recorded on acclimation of the test organism to the test dilution water, and source and pretreatment of the dilution water. Information is included on use of control test specimens in the bioassay and on statistical data treatment of bioassay results.

Bioassay data included in the CETIS are evaluated and assigned a review code value for quality, based on the experimenter's description of bioassay test methods used and on the thoroughness of the experimenter's documentation of procedures and results. Published reports meeting the following criteria are rated highest:

- Effluent collected less than 24 hours prior to testing. The method of collection was reported. Experimental procedures followed published methods.

- Standard test water chemistry data (that is, D.O., pH, temperature, alkalinity, and hardness) were reported.

- Control mortality (or other adverse effect) was satisfactory (that is, equal to or less than 10%).

- Statistical methodology used to determine the endpoint was reported.

TABLE COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM: TEST SPECIES

Species No.	Latin Name	Common Name
1	PIMEPHALES PROMELAS	FATHEAD MINNOW
2	LEPOMIS MACROCHIRUS	BLUEGILL
3	SALVELINUS FONTINALIS	BROOK TROUT
4	SALMO GAIRDNERI	RAINBOW TROUT, DONALDSON TROUT
5	DAPHNIA MAGNA	WATER FLEA
6	GAMMARUS LACUSTRIS	SCUD
7	GAMMARUS FASCIATUS	SCUD
8	DAPHNIA PULEX	WATER FLEA
10	CARCINUS MAENAS	SHORE OR GREEN CRAB
11	CRANGON CRANGON	COMMON SHRIMP
16	GAMBUSIA AFFINIS	MOSQUITOFISH

TABLE 1 (cont'd) COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM: TEST SPECIES

Species No.	Latin Name	Common Name
18	SEMOTILUS ATROMACULATUS	CREEK CHUB
19	LAGODON RHOMBOIDES	PINFISH
20	ICTALURUS PUNCTATUS	CHANNEL CATFISH
21	CYPRINUS CARPIO	COMMON, MIRROR, COLORED, CARP
22	ONCORHYNCHUS TSHAWYTSCHA	CHINOOK SALMON
23	ONCORHYNCHUS KISUTCH	COHO SALMON
25	CARASSIUS AURATUS	GOLDFISH
27	GAMMARUS PSEUDOLIMNAEUS	SCUD
28	POECILIA RETICULATA	GUPPY
30	LEPOMIS CYANELLUS	GREEN SUNFISH
33	FISH	FISH
38	PERCA FLAVESCENS	YELLOW PERCH
39	PALAEMONETES KADIAKENSIS	GRASS SHRIMP, FRESHWATER PRAWN
49	SALMO TRUTTA	BROWN TROUT
52	HYALELLA AZTECA	SCUD
54	SIMOCEPHALUS SERRULATUS	WATER FLEA
56	ASTERIAS RUBENS	STARFISH
67	CRASSOSTREA VIRGINICA	AMERICAN OR VIRGINIA OYSTER
68	SALMO SALAR	ATLANTIC SALMON
69	LABIDESTHES SICCULUS	BROOK SILVERSIDE
70	DAPHNIA SP	WATER FLEA
74	PENAEUS AZTECUS	BROWN SHRIMP
75	PENAEUS DUORARUM	PINK SHRIMP
77	PENAEUS SETIFERUS	WHITE SHRIMP (AMERICA)
82	SALVELINUS NAMAYCUSH	LAKE TROUT, SISCOWET
83	STIZOSTEDION VITREUM VITREUM	WALLEYE
85	CHIRONOMUS TENTANS	MIDGE
86	CYPRINODON VARIEGATUS	SHEEPSHEAD MINNOW
89	MICROPTERUS SALMOIDES	LARGEMOUTH BASS
90	ONCORHYNCHUS NERKA	SOCKEYE SALMON
94	BARBUS TICTO	TWO SPOTTED, TIC TAC TOE BARB
96	LEPOMIS MICROLOPHUS	REDEAR SUNFISH
102	PROCAMBARUS CLARKII	RED SWAMP CRAYFISH
105	UMBRA PYGMAEA	EASTERN MUDMINNOW
106	MICROPTERUS DOLOMIEUI	SMALLMOUTH BASS
110	LEIOSTOMUS XANTHURUS	SPOT
112	NOTEMIGONUS CRYSOLEUCAS	GOLDEN SHINER
113	CLARIAS BATRACHUS	WALKING CATFISH
133	ICTALURUS MELAS	BLACK BULLHEAD
140	CYMATOGASTER AGGREGATA	SHINER PERCH
142	MYSIDOPSIS BAHIA	OPOSSUM SHRIMP
202	LABEO ROHITA	ROHU
205	OSCILLATORIA LIMNETICA	BLUE-GREEN ALGAE
208	MYTILUS EDULIS	COMMON BAY MUSSEL, BLUE MUSSEL
211	ALBURNUS ALBURNUS	BLEAK
219	ONCORHYNCHUS GORBUSCHA	PINK SALMON
224	NEREIS ARENACEODENTATA	POLYCHAETE

TABLE 1 (cont'd) COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM: TEST SPECIES

Species No.	Latin Name	Common Name
235	CAMPELOMA DECISUM	BROWN MYSTERY SNAIL
239	PHYSA INTEGRA	POUCH SNAIL
263	COREGONUS CLUPEAFORMIS	LAKE WHITEFISH
283	CATOSTOMUS COMMERSONI	WHITE SUCKER
285	ICTALURUS NEBULOSUS	BROWN BULLHEAD
288	CULAEA INCONSTANS	BROOK STICKLEBACK
290	LEPOMIS MEGALOTIS	LONGEAR SUNFISH
293	ETHEOSTOMA SPECTABILE	ORANGETHROAT DARTER
302	PALAEMONETES PUGIO	GRASS SHRIMP, FRESHWATER PRAWN
308	MYSTUS VITTATUS	CATFISH
309	SKELETONEMA COSTATUM	DIATOM
346	HOMARUS AMERICANUS	AMERICAN LOBSTER
352	HETEROPNEUSTES FOSSILUS	INDIAN CATFISH
361	ORCONECTES VIRILIS	CRAYFISH
366	ARTEMIA SALINA	BRINE SHRIMP
371	CYPRINODONTIDAE	KILLIFISH, TOPMINNOW FAMILY
375	MENIDIA MENIDIA	ATLANTIC SILVERSIDE
376	ACARTIA TONSA	CALANOID COPEPOD
407	NOTOPTERUS NOTOPTERUS	FEATHERBACK
418	CHANNA PUNCTATUS	SNAKE-HEAD CATFISH
422	CIRRHINUS MRIGALA	CARP, HAWKFISH
423	BREVOORTIA TYRANNUS	ATLANTIC MENHADEN
436	BOWMANIELLA DISSIMILIS	MYSID, OPOSSUM SHRIMP
466	NOTROPIS CORNUTUS	COMMON SHINER
482	ALGAE	ALGAE, PHYTOPLANKTON, ALGAL MAT
483	CHAOBORUS PUNCTIPENNIS	PHANTOM MIDGE
486	SELENASTRUM CAPRICORNUTUM	GREEN ALGAE
488	INVERTEBRATES	INVERTEBRATES
508	CERATOPHYLLUM DEMERSUM	COON-TAIL
522	RANGIA CUNEATA	COMMON RANGIA OR CLAM
540	HYDROPSYCHE SP	CADDISFLY
549	HIPPOLYTE SP	SHRIMP OR PRAWN
572	CATLA CATLA	CATLA
574	ANGUILLA ANGUILLA	COMMON EEL
575	CRASSOSTREA GIGAS	PACIFIC OYSTER
578	ALOSA AESTIVALIS	BLUEBACK HERRING
602	ORCONECTES PROPINQUUS	CRAYFISH
639	OPHIOCEPHALUS PUNCTATUS	SNAKEHEAD
677	PTERONARCYS SP	STONEFLY
713	MYSTUS SEENGHALA	CATFISH
736	ONCORHYNCHUS KETA	CHUM SALMON
892	ICTALURUS NATALIS	YELLOW BULLHEAD
894	CLUPEA HARENGUS PALLASI	PACIFIC HERRING
964	CHIRONOMUS RIPARIUS	MIDGE
970	AMBYSTOMA OPACUM	MARBLED SALAMANDER
988	BUCCINUM UNDATUM	LARGE WHELK

TABLE 1 (cont'd) COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM: TEST SPECIES

Species No.	Latin Name	Common Name
1022	CTENODRILUS SERRATUS	POLYCHAETE
1074	NOTROPIS HETEROLEPIS	BLACKNOSE SHINER
1132	NOTROPIS ANOGENUS	PUGNOSE SHINER
1133	NOTROPIS EMILIAE	PUGNOSE MINNOW
1137	TUBIFEX SP	TUBIFICID WORM
1140	CENTRARCHIDAE	SUNFISH FAMILY
1178	PAROPHRYS VETULUS	ENGLISH SOLE
1186	BRANCHIURA SOWERBYI	OLIGOCHAETE
1295	MYSIDOPSIS ALMYRA	OPOSSUM SHRIMP
1321	DAPHNIA SCHODLERI	WATER FLEA
1329	HEMIGRAPSUS SP	SHORE CRAB
1369	BALANUS GLANDULA	ROCK BARNACLE
1407	LEMNA SP	DUCKWEED
1527	HETERANDRIA FORMOSA	LEAST KILLIFISH
1570	OPHRYTROCHA LABRONICA	POLYCHAETE
1584	BARBUS SOPHORE	TWO SPOTTED BARB, DOTTED BARB
1585	CHANNA MARULIUS	SNAKE-HEAD CATFISH
1586	ANISOGAMMARUS PUGETTENSIS	SCUD
1587	LEPOMIS SP	SUNFISH
1593	LEMNA PERPUSILLA	DUCKWEED
1619	POMOXIS SP	CRAPPIE
1620	HELIODIAPTOMUS VIDUUS	CALANOID COPEPOD
1738	NOTROPIS PERCOBROMUS	FISH
1739	NOTROPIS ZONATUS	BLEEDING SHINER
1905	MACROBRACHIUM KISTNENSIS	SHRIMP
1984	MYSIDACEA	MYSID OR OPOSSUM SHRIMP ORDER
1990	HYBOGNATHUS PLACITUS	PLAINS MINNOW
1991	GLYPTOTENDIPES SP	MIDGE
2052	MERCENARIA CAMPECHIENSIS	SOUTHERN QUAHOG
2053	DONAX VARIABILIS TEXASIANA	COQUINA
2054	DINOPHILUS SP	BRISTLE WORM
2065	PHOXINUS SP	DACE
2107	PAGURUS BERNHARDUS	HERMIT CRAB
2108	GAMMARUS DAIBERI	SCUD
2109	NEOMYSIS AMERICANA	OPOSSUM SHRIMP
2123	ONCHIDORIS FUSCA	SEA SLUG, NUDIBRANCH
2124	CANCER PAGURUS	EDIBLE OR ROCK CRAB
2143	MENIDIA PENINSULAE	TIDEWATER SILVERSIDE
2190	MUDFISH	MUDFISH
2191	SALVELINUS ALPINUS	ARCTIC CHAR
2203	PSAMMECHINUS MILIARIS	SEA URCHIN

The second highest data quality rating is given to published reports that contain satisfactory effluent collection methods and experimental procedures but:

- Standard test water chemistry data (D.O., pH, temperature, alkalinity, and hardness) were either only partially reported, or not reported at all.

- Control mortality or other adverse effect was not reported, was high (greater than 10 percent) or was high but accounted for statistically.

- Statistical methodology, used to determine the endpoint was not reported.

The third highest data quality rating is given to unpublished reports (such as state and Federal agency studies) that document experimental procedures and results. The lowest quality rating is given to unpublished data sheets that contain the raw test data and include minimal information about experimental procedures.

Bioassay test results may have different endpoints, and all of these are accommodated within the CETIS. For example, test results may be expressed as median lethal concentration (LC50), median lethal time (LT50) values, or median effective concentration (EC50). EC50 values may be based on such effects as abnormalities, growth, immobilization, or reproduction. Effect endpoints used are listed in Table 2.

Any additional pertinent and useful information provided by the experimenter may be included in the data base as special observations. This often includes such items as use of an effluent carrier or solvent, whether test organisms were fed or unfed, or any observations of unusual behavior or evidence of stress.

TABLE 2. EFFECT ENDPOINTS USED IN COMPLEX EFFLUENTS BIOASSAYS

ABD - Abundance: number of organisms of the same species has changed within a population

ABN - Abnormalities: physical deviations observed from normal control organisms

AVO - Avoidance: organism avoids or is attracted to certain effluent concentrations.

BEH - Behavior: quantifiable change in activity that arose from exposure to internal or external stimuli

BIO - Biochemical Effect: physiochemical reactions (e.g., change in glycogen levels) occurring within the organism on a cellular level

CYT - Cytogenetic Effect: genetic mutation on a cellular level

DIS - Disease: impairment of vital functions observed as a result of effluent concentrations, specific infective agents, inherent organism defects, or a combination of these factors

ENZ - Enzyme Effect: deviations in enzyme activity

FCR - Food Consumption Rate: quantifiable change in rate of food consumed by test animal

GRO - Growth: measured increase of animal size in length and/or weight

HAT - Hatchability: percent hatch

HEM - Hematological Effect: changes in the blood parameters observed
HIS - Histological Effect: indicated by the presence of lesions or other damage to tissues
MOR - Mortality - percentage of dead organisms
MOT - Motility: change in locomotor behavior
OC - Oxygen Consumption: change in O_2 uptake in animals
PGR - Population Growth: increase or decrease in growth of an algal population (e.g., change in cell number)
POP - Population: change in the species composition or diversity
PSE - Photosynthesis Effect: change in plant productivity
RES - Respiratory Rate: change in respiratory rate of vertebrates, invertebrates
RSD - Residue: toxicant uptake by tissues of test organism
SS - Swimming Speed: change in swimming speed
STR - Stress: observed physiological tension in animals or plants
TMR - Tumor Occurrence: presence of a mass of abnormal tissue

---

RESULTS

Data from 500 references for 1500 bioassay tests are in the CETIS data base as of June 1984. These comprise data from 140 published papers (2050 tests) and from 900 state data sheets (2600 tests). The data currently include information from 16 states in the United States and from Canada.

Entered data also are transmitted to a larger computer system at the National Computer Center in Raleigh, North Carolina. These data are cross-referenced with corresponding data from other data bases at the National Computer Center. These are the Industrial Facilities Discharge (IFD) file, which is linked to the United States Geological Survey river gage level file (GAGE) by way of a hydrologic network file (REACH). By these linkages, cross-referenced information from the Industrial Facilities Discharge file, river gage level file, and hydrologic network file are included in the CETIS data base. Figures 1 and 2 provide schematic diagrams of the overall data base management system, showing the ERL-Duluth and National Computer Center portions.

Computer programs have been prepared to search and sort through the data base to retrieve compiled information. These template retrievals are available to provide data selected by industry, area or receiving water, test, test species, and effluent treatment. A total data listing for up to eight references can also be obtained. Table 3 provides a summary of retrieval options. Figure 3 is an example of a template retrieval output sorted by test. Users can also design and implement specific retrievals of their own design to obtain data from the system. A user's guide and programmer's manual describe these programs in detail (Gueldner et al. 1984). Development of programs is underway to analyze the effluent toxicity data to provide comparisons between organisms, effluents, and bioassay endpoints.

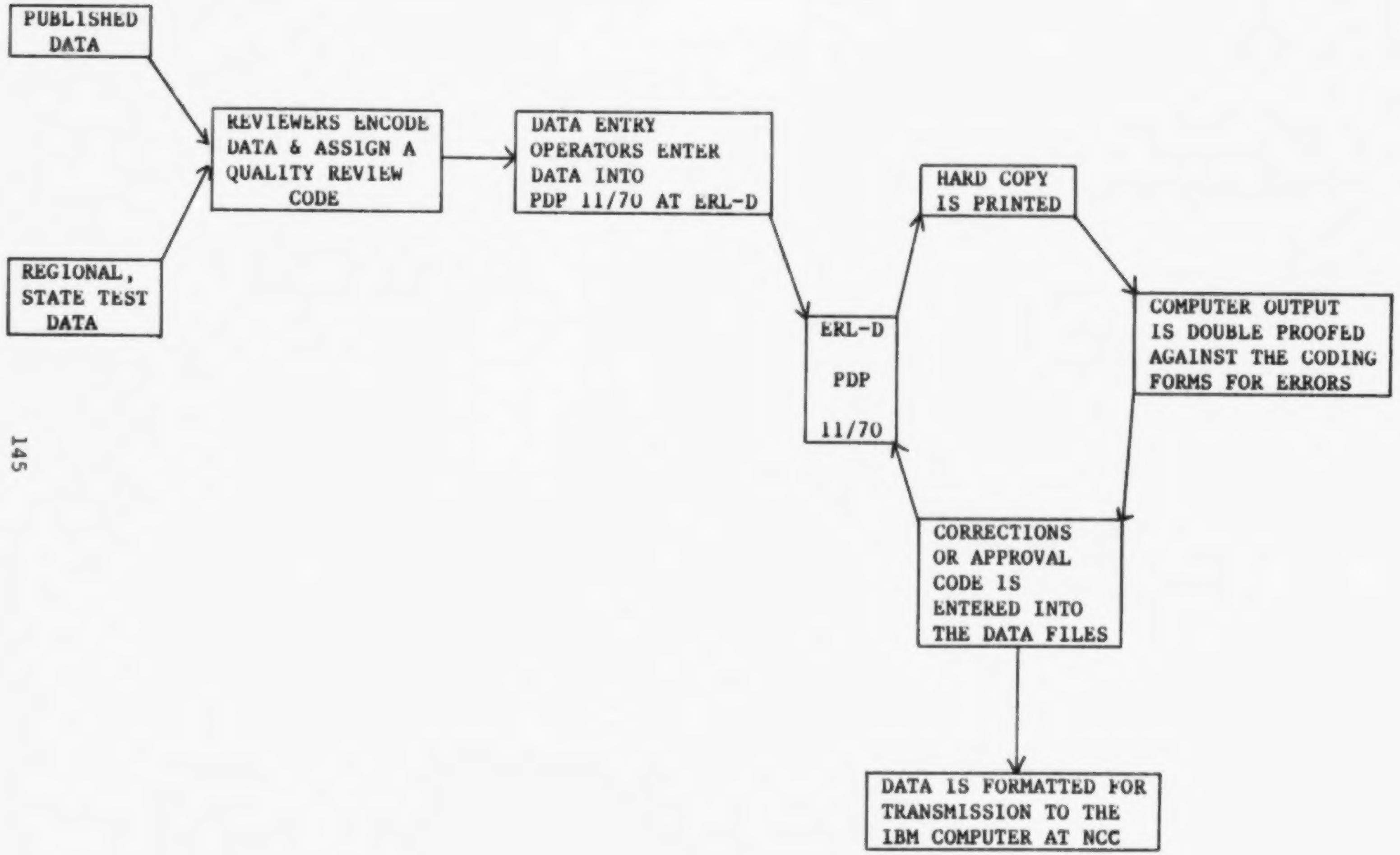

PUBLISHED DATA
REGIONAL, STATE TEST DATA
REVIEWERS ENCODE DATA & ASSIGN A QUALITY REVIEW CODE
DATA ENTRY OPERATORS ENTER DATA INTO PDP 11/70 AT ERL-D
ERL-D PDP 11/70
HARD COPY IS PRINTED
COMPUTER OUTPUT IS DOUBLE PROOFED AGAINST THE CODING FORMS FOR ERRORS
CORRECTIONS OR APPROVAL CODE IS ENTERED INTO THE DATA FILES
DATA IS FORMATTED FOR TRANSMISSION TO THE IBM COMPUTER AT NCC

Figure 1. CETIS at ERL-Duluth

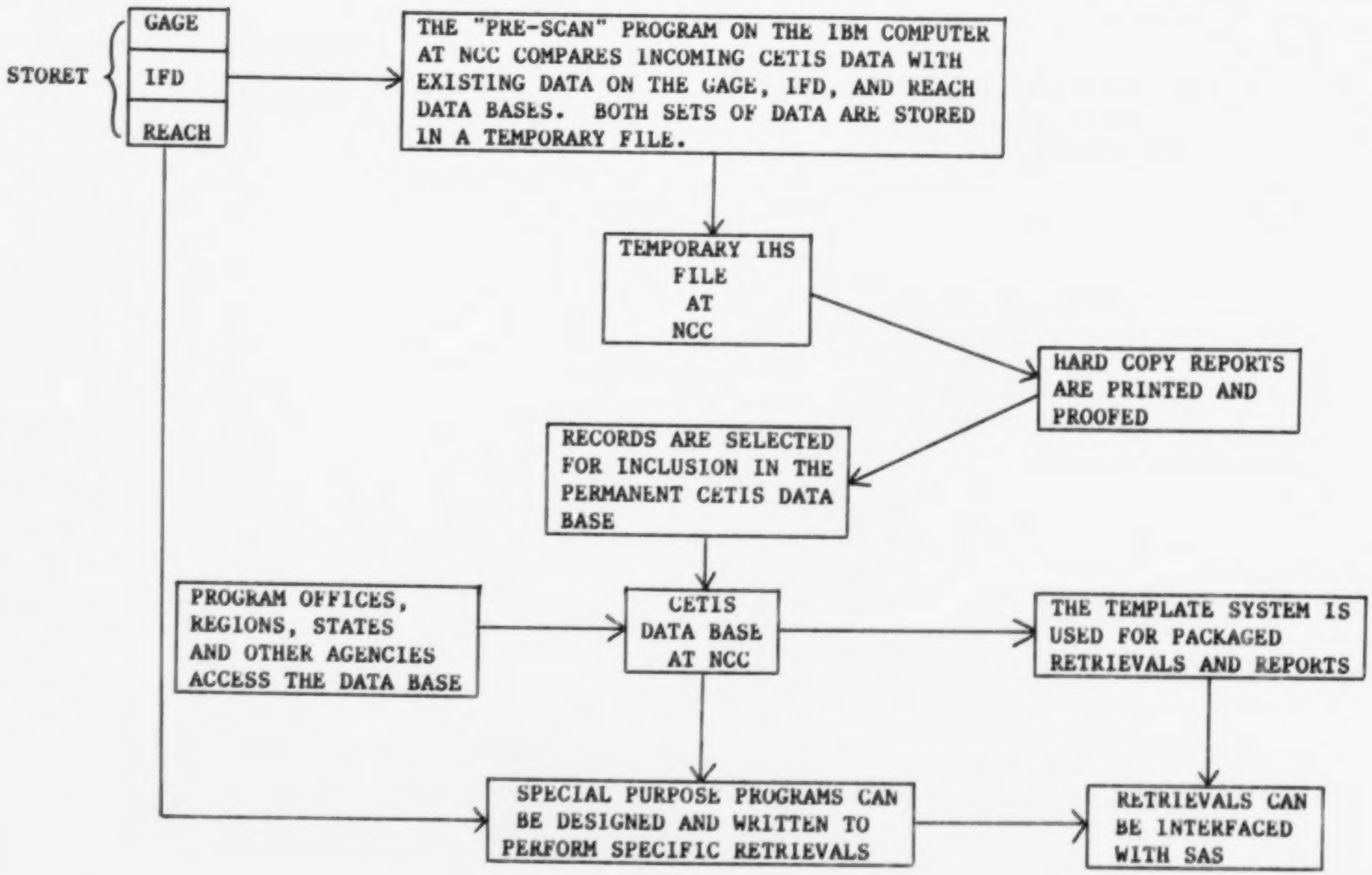

STORET
GAGE
IFD
REACH
THE "PRE-SCAN" PROGRAM ON THE IBM COMPUTER AT NCC COMPARES INCOMING CETIS DATA WITH EXISTING DATA ON THE GAGE, IFD, AND REACH DATA BASES. BOTH SETS OF DATA ARE STORED IN A TEMPORARY FILE.
TEMPORARY IHS FILE AT NCC
HARD COPY REPORTS ARE PRINTED AND PROOFED
RECORDS ARE SELECTED FOR INCLUSION IN THE PERMANENT CETIS DATA BASE
PROGRAM OFFICES, REGIONS, STATES AND OTHER AGENCIES ACCESS THE DATA BASE
CETIS DATA BASE AT NCC
THE TEMPLATE SYSTEM IS USED FOR PACKAGED RETRIEVALS AND REPORTS
SPECIAL PURPOSE PROGRAMS CAN BE DESIGNED AND WRITTEN TO PERFORM SPECIFIC RETRIEVALS
RETRIEVALS CAN BE INTERFACED WITH SAS

Figure 2. CETIS at National Computer Center

TABLE 3. CETIS REPORT SPECIFICATION SUMMARY RETRIEVAL OPTIONS

Report Options	Select Options	Sort Options	Format Options
Industry Report	Industrial Category SIC Codes*	SIC Codes & State State & SIC Codes	Brief Format Expanded Format SAS Format Disk File
Area or Receiving Water Report	Eight-digit Catalog Unit Eight-digit Catalog Unit & three-digit Segment Number Basin Code State	Catalog Unit & Segment Number NPDES Number†	Brief Format Expanded Format SAS Format Disk
Test Report	Bioassay Type 1 Bioassay Type 2 Exposure Type Test Duration	NPDES Number SIC Code	Brief Format Expanded Format SAS Format Disk File
Test Species Report	Test Species Lifestage	NPDES Number SIC Code	Brief Format Expanded Format SAS Format Disk File
Effluent Treatment Report	NPDES Number SIC Code Effluent Treatment	NPDES Number SIC Code Low Flow Discharge Flow	Brief Format Expanded Format SAS Format Disk File
Total Data Listing Report	Reference Numbers	Not applicable	Full Data Listing Format

---

*SIC code = Standard Industrial Classification Code

†NPDES number = National Pollutant Discharge Elimination System Number (discharge number)

```
=======================================================================================================================
REFERENCE #     SIC
                          EXP.    BIO.   BIO.    TEST
   TEST DATE    TEST #    TYPE    TYP-1  TYP-2   DUR.    SPECIES   CONC.     CONC. RANGE     EFFECT
                                                                                  UP       PH        TEMP      ALK       HARD
=======================================================================================================================
EHL00738500     4952
     /  /           1      F       A      C       84       23       1.1
     /  /                                         84                3.6
     /  /                                        112                1.1
     /  /                                        112                3.6

GAFL00009000    4952                                                             .7                13.7    106.7
    80/06/25
                    1      S       A      C        1        1        36
                                                                                           LC50
    80/06/25        2      S       A      C        1        8        47                 6.4                 125        100
                                                                                           LC50
MIED00011000    4952                                                                    6.4           22    125        100
    74/11/18
                    1      F       A      C        4        1       1.7
                                                                                           LC50

OHED00017000    4952
    75/05/30        1              A      C        4        1        45
                                                                                           LC50
    75/08/21        2              A      C                 1       100                  7            21               260

    73/11/21        3      S       A      C        4        1       100                               21

     /  /           4      S       A      C        2        8       100                  7            21    315        262

OHED00018000    4952                                                                     7            21    315        262
    76/03/03        1              A      C        4        1       100

OHED00054000    4952                                                                   7.6            21
    73/11/12        1      S       A      C        4        1        13
                                                                                           LC50
    73/11/12        2      S       A      C        2        7        26                 7.5            21               242
                                                                                           LC50
OHED00056000    4952                                                                   7.5            21               242
    73/10/18        1      S       A      C        4        1       100

    73/10/18        2      S       A      C        2        1        85                 7.4            21                82
                                                                                           LC50
OHED00057000    4952                                                                   7.4            21                82
    73/10/23        1      S       A      C        4        1       100

    73/11/01        2      S       A      C        2        8        83                 7.2            21               238
                                                                                           LC50
OHED00058000    4952                                                                   7.2            21               238
    73/10/24        1      S       A      C        4        1       100

    73/11/01        2      S       A      C        2        8       100                 7.3            21               200

OHED00059000    4952                                                                   7.3            21               200
    73/10/30        1      S       A      C        4        1        42
                                                                                           LC50
    73/10/30        2      S       A      C        2        7        32                 7.6            21               304
                                                                                           LC50
                                                                                       7.6            21               304
```

Figure 3. Example of CETIS retrieval system test report--expanded format

ACKNOWLEDGMENT

Many individuals have been involved with the Complex Effluents Toxicity Information System, and their contributions are gratefully acknowledged. Bruce Newton has provided invaluable suggestions on data base development. Kenneth Carlson, Dorette Gueldner, Charles Marks, Jeanne Rondeau, Daniel Sivertson, and Phillip Taylor contributed to programming tasks. Cay Moriarity, Beth Nordling, and Eve Katich assisted with reviewing and encoding of data. Dorette Gueldner and Judy Veith have worked as data entry operators. PEDCO Environmental, Inc., assisted with reviewing, encoding, and entering data.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1981. Standard methods for the examination of water and wastewater, 15th ed. Am. Public Health Assoc., New York. 1134 p.

American Society for Testing and Materials. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. pp. 1-25 in Annual Book of ASTM Standards.

Gueldner, D.R., A. Pilli, J.L. Crane, and D.J. Sivertson. 1984. CETIS: Complex Effluents Toxicity Information System. CETIS retrieval system user's manual. EPA/600/8-84-030, U.S. Environmental Protection Agency, Duluth, Minnesota. (In press). 10 p.

Gueldner, D.R., D.J. Sivertson, and A. Pilli. 1984. CETIS: Complex Effluents Toxicity Information System. Programmer's manual. U.S. Environmental Protection Agency, Duluth, Minnesota. (Unpublished report). 92 p.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. U.S. Environmental Protection Agency. Cincinnati, Ohio. EPA-600/4-78-012.

Peltier, W., and C.I. Weber. 1984. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/4-85/013.

U.S. Environmental Protection Agency. 1971. Algal assay procedure bottle test. Nat. Eutrophication Res. Pro. 82 p.

Weber, C.I., and W. Peltier. 1981 Effluent toxicity screening test using daphnia and mysid shrimp. U.S. Environmental Protection Agency, Cincinnati, Ohio. (Unpublished report.)

OPTIMIZING A PROGRAM FOR THE DEVELOPMENT OF WATER CONSERVATION IN A SYSTEM OF WATER QUALITY MANAGEMENT CONSIDERING POINT AND NON-POINT POLLUTION SOURCES

by

G. A. Sukhorukov[1]

ABSTRACT

A mathematical model is examined for optimizing a program of gradual and coordinated development of water conservation measures for achieving standard water quality. The model includes a unit for the development of new production methods, a unit for the development of procedures for processing and recovering waste waters, including the establishing of intersectorial and regional reusable systems, and a unit for forming water quality.

For selecting the optimum program for gradually achieving standard water quality, a quadratic criterion has been proposed for the accuracy of achieving the maximum tolerable effluents. For considering the impact of non-point pollution sources and for calculating the maximum acceptable effluents, two approaches have been examined--determined and probability. A system and model also have been proposed for calculating the optimum water conservation measures according to the criterion of the minimum mathematical expectation of the loss functions.

INTRODUCTION

Long-term and current forecasting and planning of water-protection measures are tasks of utmost importance in the control of water quality. Unlike ongoing control, where such parameters as capacity of industrial and water sources are considered to be relatively stable, long-term and current planning includes such parameters as the basic controllable variables. Thus, long-term and current planning tasks determine the general task of controlling development of water protection measures.

The complexity of developing water protection measures is the reason for the urgency and necessity of developing mathematical models for optimizing development of industrial and water protection complexes, and adopting wide use of computers.

---

[1] All-Union Scientific Research Institute of Water Protection, USSR Ministry of Land Reclamation and Water Resources, Kharkov, USSR

Analysis of the survey literature (Vavilin and Tsitkin 1977) and other sources shows that formulation of the tasks of optimum development of water protection was based on optimization of parameters of treatment plants as the basic water protection measure. In a number of instances, such tasks included optimization of water drawdown. As a rule, such tasks were static (single step) and did not include units for optimization of production technology, which is the main factor that influences the quantity and composition of diverted water prior to purification.

The urgency of the problem of coordinated development of production technology and treatment (purification) technology and the use of diverted water had been repeatedly discussed in the literature; several models with a high unitization level determining the correlation between development of industry and environmental protection were proposed (Anon. 1982). At the same time, there is still the pressing problem of developing mathematical models and methods of optimizing coordinated development of technology of production, processing, and utilization of diverted water and water-use tehnology on the level of an individual enterprise or a set of enterprises united by intersector water management systems.

It is expressly on this level, for enterprises, that the key parameters are determined: maximum permissible discharge of impurities into water projects, plans for development of industry, and water protection measures consistent with the plans for construction of installations. On the basis of the foregoing, we are considering models for optimization of a set of water protection measures as a set of models for optimization of industry and its development (Industry unit), models for optimization of treatment and use of diverted water (Treatment and utilization unit), and models of processes within water reservoirs (Water system).

The dynamic nature of development of industry and water protection, the multistage nature of development of industry and water protection, and the multistage planning of such development (in 5-year periods) determine the need to develop multistep, dynamic models of optimizaton of programs for coordinated development of industrial and water protection complexes. The inherent distinction of such models is that there are intermediate and end goals of development and criteria of optimality for effectiveness of achieving these goals stage by stage.

Bearing in mind the above-mentioned distinctions of optimization of the set of water protection measures, we developed a system of optimization models for coordinated development of industry and water protection, which is based on the method of building dynamic models of developing systems (Sukhorukov 1977).

Such models are designed for use in developing current and future plans and forecasts. In such developments, the choice of methods of considering nonpoint sources of pollution--surface runoff from cities and farms--is of substantial significance. This must also be reflected in the models of optimization of programs for development of water protection, and the problem arises of considering the random nature of processes of removal of superficial runoff.

MODELS FOR OPTIMIZATION OF PROGRAMS OF DEVELOPMENT OF WATER PROTECTION

Let us examine models for optimization of developing systems of technological complexes. We shall use the term, technological complex (TK), to refer to the interrelated aggregate of units (elements of complex) that affect physical, energetic, and informational conversion in order to obtain a specified end result of production.

A developing system will be considered given (specified) if the following has been determined:

A. Finite set of points in time or steps, T = (t) limited by interval of development [0, T], aggregate of sets, elements of which are the following non-negative phase variables (vectors): Y(t)--end results of development, Y(t=0) = Y(0)--original state Y(t), V(t)---current control; Z(t)--status of system determining maximum production capacity and its initial state Z(t=0) = Z(0), u(t)--control of system development, i.e., control of development of production capacities, β_t--external resources, ω_t--assignment beyond purpose of development.

B. Model of object of control that determines its "physical" properties, as a set of equations such as:

$$S(y,v,z,u,\beta,t)=0. \tag{1}$$

Criterion of optimality of development

$$f(y,v,z,u,\beta,t)\rightarrow \mathrm{extR}. \tag{2}$$

Set of restrictions on phasic variables:

$$u(t)\in\mathcal{U}\ ;\quad v(t)\in\mathcal{V}\ ;\quad z(t)\in\mathcal{Z}, \tag{3}$$

- resources $\beta_t\in\mathcal{B}$, (4)

- goals of development $y(t)\in\Omega$. (5)

In these expressions, we use the term, program of development, to refer to the aggregate of vectors of phasic variables X(t) = [Y(t), V(t), Z(t), U(t)].

In essence, optimization of development programs consists of selecting set x(t) on t ε T, with which the system changes from base state Z(0) to states Z(1)...Z(t)...Z(T) as a result of controlling development--u(1)... u(t)...u(T)--so that current equations--v(1)...v(t)...v(T)--lead to achievement of end results--y(1)...y(t)...y(T), which meet externally given goals of development (5), provided the extreme is reached for the given criterion of optimality of development (2). The set of restrictions (1, 3, 4) that determine the correlation between phasic variables and restrictions for each of them (3), as well as restrictions on resources (4) allocated for development (capital investments, etc.)--$\beta_1 \ldots \beta_t \ldots \beta_T$--that are considered given, are also important to choice of optimum program.

Proceeding from the general model of optimization of development (1-5) and models of special-purpose programmed planning, let us formulate a typical problem of optimization of program of development of technology of the complex in the following rather simple form:

$$\left\{ f(v,u) = \sum_t f_v(v(t)) + \sum_t{}' f_u(u(t)) \right\} \to \min, \tag{6}$$

$$B v(t) = y(t), \tag{7}$$

$$D v(t) \leq z(t), \tag{8}$$

$$z(t) = z(t-1) + u(t), \tag{9}$$

$$y(t) \in \Omega; \; v(t) \in V; \; u(t) \in U; \; z(t=0) = z(0), \tag{10}$$

where fv and fu are operating and capital expenditures with consideration of adduction, B is matrix of direct expenses, D is matrix of production capacity expenses, y is vector of end product, v is vector of total product (current production control), z is vector of production capacity, u is vector of control of development of production capacity, Ω is the set of given end goals, V and U are sets detemining restrictions on v(t), u(t) and t ε T, z(0) is the initial state of production capacity with consideration of equipment no longer used.

Solution of problems (6-10) yields the optimum program for ongoing control $\overset{\circ}{v}(t)$ and development $\overset{\circ}{u}(t)$ of the technological state with consideration of its base state z(0) and need to achieve intermediate and end goals w_t in interval [1, T].

Let us turn to construction of a model for optimization of development of a set of water protection measures for a basin (region). We shall proceed from the following premises.

1. The model defines the set of measures directed toward improvement and development of technology of production, technology of treatment and utilization of diverted water with consideration of development of intersector water-management systems, and technology of water use related to increase in assimilating capacity of the water system in question (as a result of drawdown from reservoirs, diversion of run-off, etc.). Accordingly, three units are distinguished in the model--"Industry," "Treatment and utilization," "Water system."

2. The tasks of releasing the end product and locating the plant are considered to be given for planning intervals, since their estimation is made on a higher level in the hierarachy of national economic planning.

3. Standards for water quality considered set and tied in with given specified check section lines, which is determined by the type of water use on a river section.

4. The water-consuming enterprise, which is characterized by the following elements, is an elementary subsystem of the model: discharge of liquid waste, unit with given technology of production or treatment of diverted water.

5. The overall task of optimizing development is separated into two tasks, which are, in general terms, as follows:

> Optimization of development of set of water protection measures for the entire planning period [0, T], consisting of more than one 5-year interval according to criterion of minimal adduced expenditures in the absence of restrictions on external resources.
>
> Optimization of development of set of water protection measures for the first and subsequent 5-year periods in the presence of restrictions on overall capital investments, according to criterion of minimal Δ between given values for maximum permissible discharge (MPD) of substances for which standards are set (in each enterprise) and level of obtained discharge of substances, values of MPD are found by solving the first problem.

Each pair of problems is solved every 5 years with a shift made so that MPD is recalculated in every 5-year period.

Let us consider the task of optimizing development of the set of water protection measures for the period [0, T] for a set of enterprises $\kappa \varepsilon K$, which contains the following components.

Criterion of optimality--overall adduced expenses:

$$\left\{ \sum_{K}{}' \left(f_K^{(1)}(u_K^{(1)}, V_K^{(1)}) + f_K^{(2)}(u_K^{(2)}, V_K^{(2)}) \right) + \sum_{\ell \in K^{(3)}}{}' f^{(3)}(u_\ell^{(3)}, V_\ell^{(3)}) \right\} \to \min_{u,V}, \qquad (11)$$

I. "Industry" unit--κth enterprise, $\kappa \varepsilon K$

$$B_{\kappa}^{(1)} V_{\kappa}^{(1)} = \omega_{\kappa}^{(1)}(T), \tag{12}$$

$$D_{\kappa}^{(1)} V_{\kappa}^{(1)} - u_{\kappa}^{(1)} \leq Z_{\kappa}^{(1)}(0, T), \tag{13}$$

$$u_{\kappa}^{(1)} \in U_{\kappa}^{(1)}, \tag{14}$$

where $B^{(1)}$ is the technological matrix of production balance, $D^{(1)}$ is matrix of coefficients of specific expenditures of unit output, $V^{(1)} = (V_{i}^{(1)})$ is total output of the ith type of product on the θ_1th unit or by the θ_1th technology. $U^{(1)} = (U_{\theta_1}^{(1)})$ is the vector of buildup of production capacity of the θ_1th unit, $Z_{\kappa}^{(1)}(0, T) = (Z_{\theta_1}^{(1)})$ is the vector of initial status of production capacity of the θ_1th unit with consideration of removal of capacities from use, $U_{\kappa}^{(1)}$ is the set determining specific restrictions on control $u_{\kappa}^{(1)}$.

II. "Treatment and utilization" unit, κth enterprise, $\kappa \varepsilon K$ equations of balance of consumption of fresh, recycled and reused water, balance of water diversion

$$M_{\kappa} V_{\kappa}^{(1)} - \mathcal{E}_{\kappa}^{\overline{I}} V_{\kappa}^{(2)} - \mathcal{E}_{\kappa}^{\overline{II}} p_{\kappa} = 0, \quad (P_{\kappa} \leq \bar{P}_{\kappa}) \tag{15}$$

$$G_{\kappa}^{(1,2)} V_{\kappa}^{(1)} - \mathcal{E}_{\kappa}^{\overline{III}} V_{\kappa}^{(2)} + \mathcal{E}_{\kappa}^{\overline{IV}} m_{\kappa} = 0, \tag{16}$$

where M_{κ} is the matrix of standards of water consumption according to category (fresh water, industrial), $G^{(1,2)}$ is matrix of standards of water diversion (according to categories of run-off), ε^{I}, ε^{II}, ε^{III}, ε^{IV} are zero-unit [?] structural matrices, $V_{\kappa}^{(2)}$ is vector of outlay of diverted water treated via different technological routes $s \varepsilon S$. Set S_{κ} is broken down into the following nonintersecting subsets: S_1^{κ} treatment and discharge into water system, S_2^{κ} recycled and reused for own use, S_3^{κ} treatment and transfer to other enterprises; $P_{\kappa} = (P_1^{\kappa}, P_2^{\kappa})$ is outlay of consumed fresh water, including P_1^{κ} from superficial sources and P_2^{κ} from subterranean sources; $\bar{P}_{\kappa}$ is limit on water consumption and m_{κ} is outlay of water coming from other enterprises.

$$m_{\kappa} = \sum_{\gamma \neq \kappa} \sum_{s \in S_3^{\gamma}} V_{\gamma, s}^{(2)}, \quad \gamma \in K_n \tag{17}$$

where Kn is set of enterprises situated in the same industrial region or city where it is feasible to organize intersector water-management systems, system of inequalities that take into consideration development of production capacity of the "Treatment and utilization" unit.

$$D_{\kappa}^{(2)} V_{\kappa}^{(2)} - u_{\kappa}^{(2)} \leq Z_{\kappa}^{(2)}(0, T), \tag{18}$$

$$u_{\kappa}^{(2)} \in U_{k}^{(2)} \tag{19}$$

where D is the matrix of coefficients of specific expenditures of production capacity of units, $u_{\kappa}^{(2)} = (U_{\theta 2}^{(2)})$ is vector of buildup of production capacity, $Z^{(2)}(0, T) = Z_{\theta 2}^{(2)})$ is the vector of initial status of production capacity of th unit with consideration of capacity no longer used.

III. "Water system" unit.

System of recurrent equations of transfer and transformation of impurities:

$$Y_{\kappa+1} = A_{\kappa+1,\kappa}^{Y} Y_{\kappa} + A_{\kappa+1,\kappa}^{V} \left[G_{\kappa}^{(2)} V_{\kappa}^{(2)} + b_{\kappa} \right], \ \kappa \in K^{\Sigma} = K, \tag{20}$$

where $\kappa+1$, κ are numbers of estimated section lines in stream, A^{y} and A^{v} are matrices of coefficients that consider dilution, self purification and transformation of impurities, which also depend on expenditure increment in the section $(\kappa+1, \kappa)$, $Y_{\kappa+1}$ is vector of concentrations of impurities for which standards are set in the water system, $G_{\kappa}^{(2)}$ is the matrix of concentrations of impurities in sewage after treatment, b_{κ} is vector of uncontrolled discharge of impurities,

$$A_{\kappa+1,\kappa}^{Y} = \mathcal{A}_{\kappa+1}^{Y}(Y_{0,\kappa+1}); \qquad A_{\kappa+1,\kappa}^{V} = \mathcal{A}_{\kappa+1}^{V}(Y_{0,\kappa+1})$$

system of restrictions on water quality in control section lines

$$\mathcal{Z} Y_{\overset{*}{\kappa}} \leq \omega_{\overset{*}{\kappa}}^{(3)}, \quad \overset{*}{\kappa} \in K^{*} \subset K^{\Sigma}, \tag{21}$$

where Z is goal matrix, $w_{\kappa}^{(3)}$ is vector of water quality standards (with consideration of LPV [expansion unknown]).

System of recurrent equations of water balance.

$$Y_{0\kappa+1} = Y_{0\kappa} + \mathcal{E}_{\kappa}^{\bar{V}} V_{\kappa}^{(2)} - P_{1}^{\kappa} + \lambda_{\kappa} + \delta Y_{0\kappa} \leq \bar{Y}_{0\kappa+1} \tag{22}$$

where $Y_{0\kappa+1}$ and $Y_{0\kappa}$ are estimated outlay of water in section lines $\kappa+1$ and κ; λ_κ is influx in the section ($\kappa+1$, κ) with consideration of water-management balance, $\delta_{Y_{0\kappa}}$ is increment of expenses due to drawdown from reservoirs, diversion of run-off, etc., $\overline{Y}_{0\kappa+1}$ is minimal environment-protective outlay, $\varepsilon_{\kappa}^{\underline{V}}$ is zero-unit structural matrix.

System of equations determining feasiblity of increasing diluting capacity of water system:

$$\sum_{\ell\in K^{(3)}} \alpha_{\kappa\ell}^{(3)} v_\ell^{(3)} = \delta_{Y_{0\kappa}}, \quad \kappa \in K^{\mathrm{I}}, \tag{23}$$

$$d_\ell^{(3)} v_\ell^{(3)} - u_\ell^{(3)} \leq z_\ell^{(3)}(0,T), \quad \ell \in K^{(3)}, \tag{24}$$

$$v_\ell^{(3)} \in V^{(3)}, \; u_\ell^{(3)} \in U_\ell^{(3)} \quad \ell \in K^{(3)}, \tag{25}$$

where $v_\ell^{(3)}$ is additional outlay from ℓth water-management system (reservior, canal) in the ℓth section line, $\alpha_{\kappa\ell}^{(3)}$ is coefficient of influence ($0 \leq \alpha^{(3)} \leq 1$), $u_\ell^{(3)}$ is increment of indicators of production capacity of ℓth water-management system, $d_\ell^{(3)}$ is coefficient of specific expenditures of production capacity of ℓth system, $V^{(3)}$ and $U^{(3)}$ are sets defining specific restrictions.

As a result of solving problems (11-25), the optimum values for $\overset{\circ}{v}(T)$ and $\overset{\circ}{u}(T)$ are calculated, which determine all the necessary water-management characteristics:

Input of capacities into "Industry," "Treatment and utilization" and "Water system" units.

Volumes (expenditures) of fresh, recycled, reused water, volumes of water discharged according to type of treatment (purification).

Capital [fixed asset] and operating expenses for implementation of integrated measures.

Maximum permitted dumping of pollutants into water systems of different enterprises.

$$\overset{\circ}{\omega}{}_{\xi\kappa}^{(2)} = {\sum_{s}}' g_{\kappa\xi s}^{(2)} \overset{\circ}{v}{}_{\kappa s}^{(2)}, \quad s \in S_1^{\kappa}, \; \xi \in \Xi, \tag{26}$$

where ξ is the type of substance for which standard is being set, $g_{\xi s}^{(2)}$ is concentration of substance in sewage after treatment via sth route (element of matrix $G^{(2)}$).

Calculation of $\mathring{W}^{(2)}_{\xi\kappa}$ is probably one of the most important results of the problem, which permit in the next step solving the set of local problems for the first 5-year planning period in order to define the priority water-protection measures. Let us consider, in order to describe formulation of such problems, a hierarchically organized system with the following elements: a) restrictions on overall capital expenditures in the basin (region) and global criterion of optimality, b) set of local systems for individual enterprises, or set of several enterprises (industrial centers, cities)--$n \in N$.

We shall use the previously introduced definitions and designations to form the optimization problem.

Global criterion of optimality is the minimum sum of squares of discrepancies determining a mismatch between values and MPD--$\mathring{W}^{(2)}_{\kappa}$ and obtained disposal of impurities for which standards are set.

$$\left\{ \Phi(\rho) = \sum_{n\in N}' \varphi_n(\rho_n) = \sum_{n\in N}' \sum_{\kappa\in K}' \rho_\kappa' R_\kappa \rho_\kappa \right\} \to \min_{\rho} \qquad (27)$$

where ζ_κ is the vector of discrepancies [errors] and R is a positively difined matrix (in particular, R = E).

Global restrictions on total capital expenditures for the basin (region are:

$$\sum_{n\in N}' \mu_n(1) \le \beta(1) \qquad (28)$$

where μ_n is scalar coordinating parameter.

Restrictions on overall capital expenditures allocated for development of the industry and water-protection measures of the nth subsystem are:

$$\sum_{\kappa\in K_n}' \left(f^{(1)}_{u\kappa}\left(u^{(1)}_\kappa(1)\right) + f^{(2)}_{u\kappa}\left(u^{(2)}_\kappa(1)\right) \right) \le \mu_n(1) \qquad (29)$$

"Industry" unit of κth subsystem, $\kappa \in K$:

$$B^{(1)}_\kappa V^{(1)}_\kappa(1) = w^{(1)}_\kappa(1) \qquad (30)$$

$$D^{(1)}_\kappa V^{(1)}_\kappa(1) - u^{(1)}_\kappa(1) \le Z^{(1)}_\kappa(0,1) \qquad (31)$$

$$u^{(1)}_\kappa \in U^{(1)}_\kappa(1) \qquad (32)$$

where $U_{k}^{(1)}(1)$ is the set of controls of development, elements of which are defined by solving the preceding problem for the period [0, T].

"Processing and utilization" unit:

$$M_k V_k^{(1)}(1) - \mathcal{E}_k^{\overline{I}} V_k^{(2)} - \mathcal{E}^{\overline{II}} P_k = 0 \qquad (\rho_k \le \overline{\rho}_k) \tag{33}$$

$$G_k^{(1,2)} V_{k(1)}^{(1)} - \mathcal{E}_k^{\overline{III}} V_{k(1)}^{(2)} + \mathcal{E}^{\overline{IV}} m_k = 0 \tag{34}$$

$$\tilde{G}_k^{(2)} V_k^{(2)}(1) - \rho_k(1) \le e_1 \tag{35}$$

$$D_k^{(2)} V_k^{(2)}(1) - u_k^{(2)}(1) \le Z_k^{(2)}(0,1) \tag{36}$$

$$u_k^{(2)} \in U_{k(1)}^{(2)} \tag{37}$$

where $\tilde{G}_k$ is the matrix, the elements of which are found by dividing concentrations of impurities (elements of matrix $G_k^{(2)}$) by MPD--$\mathring{W}_{\xi k}^{(2)}$, $U_k^{(2)}(1)$ is set of equations of controls of development, elements of which are found from solving the preceding problem for the period [0, T], and e_1 is a unit vector.

As can be seen from (27-37), the model of the water is not considered, since restrictions on impurity discharge with sewage is determined by MPD.

The problem we have discussed (27-37) contains $n \epsilon N$ local subsystems, each of which corresponds to an industrial center, city or agglomeration and urban centers and industrial enterprises, agricultural enterprises where there is a potential possibility of establishing intersector water-management systems, including general treatment plants, local and intersector recycling. Expressly such a structure of systems of treatment and utilization is inherent in all industrially developed regions, so that planning tasks should solve the actually ocurring problems of intersector cooperation. Of course, in the special case, each subsystem can contain only one enterprise.

The above tasks were defined in continuous (nonintegral) variables, which makes it possible to obtain the "absolutely best" result, as compared to integral formulation. Problems (27-37) can be solved in integral formulation, if this is necessary, by different methods. One method involves introduction of integral variables in the following manner. variables U_θ are replaced with the following expression:

$$u_\theta = \sum_{j \in J} \delta_{\theta j} \bar{u}_{\theta j}, \qquad \delta_{\theta j} = 0 \wedge 1,$$

where $\delta_{\theta j}$ is the optimizing variable, $\bar{u}_{\theta j}$ is the given numerical series of unified values for production capacities.

Another, simpler method with regard to calculations, involves finding the closest integral solution $\bar{U}_{\theta j} \geq U_{\theta}$.

On the whole, choice of problem solving method depends on the type of functional and functions contained in the system of restrictions. In the case of several simplifications, the problems can be reduced to problems of mathematical linear (LP) and convex programming with use of standard packages of applied LP programs in automated control systems. Problems (27-37) are solved on two levels using a parametric noninteractive procedure (Sukhorukov 1977).

It should be noted that, along with use of systems (11-26, 27-37) in the optimization mode, such systems can be well-used in the direct reading mode for making forecasts for the period [0, T] or any intervals [t, t-1). In addition to obtaining a practical result--for example, forecasting water quality--such a mode makes it possible to determine some initial approximation for optimization problems.

CONSIDERATION OF NONPOINT SOURCES OF POLLUTION WITH DETERMINISTIC AND PROBABILISTIC FORMULATION OF PROBLEMS

Because of the specific nature of nonpoint sources of pollution of surface run-off in cities and farms, they can be taken into consideration in optimizing water-protective measures in two ways: a) removal of impurities from non-point sources is considered uncontrollable (unoptimizable) in the general model, depending only on the characteristics of the water catchment area and precipitation, as components of hydrological calculations, b) removal of impurities also depends on the set of water protection measures for decontamination of superficial run-off, which are characterized by optimizable variables. These variables are analogous to those discussed above, and they are included in the "Industry" unit (for example, change in technology of agriculture) and "Treatment and utilization" unit (for example, treatment of a city's surface run-off).

In both the first and second instances, there can be both deterministic and probabilistic formulation of the problem.

With deterministic formulation of the problem, the system of models described above remains unchanged in structure, merely new elements (optimizable and unoptimizable) are added to the "Water system" unit (2022). The difficulty lies in estimating the unoptimizable removal of impurities with superficial run-off. In addition to other methods, this can be calculated by separating the superficial component of run-off in a river section per month from the aggregate of surface and underground runoff under specified nominal conditions, for example, in a year of 95% supply. Then, on the basis of the charateristics of the water catchment area, determination is made of mean concentration of impurities in superficial run-off and removal of impurities in water system averaged for a month. The advantages of such an approach is that removal of impurities with superficial run-off is tied in to nominal hydrological conditions, and it is possible to consider the joint effect of point and nonpoint sources on water quality under the same estimated conditions, as well as to build a deterministic model of optimization of level of decontamination of superficial run-of. A comprehensive method was developed on the basis of such an approach (Pasyuga 1985) and has been used in practice.

At the same time, the physical nature of such processes as removal of impurities with surface run-off in cities and outlay of water in the stream is in the nature of random processes with considerable deviation from mean values, which is why probabilistic approaches are relevant. In this case, to build models of optimization with consideration of random processes, an approach is proposed, in which the criterion of optimum (minimum or maximum) mathematical expectation of loss function is used as the optimality criterion, which take into consideration the probabilistic characteristics of the random process.

Let us discuss in greater detail the method of building optimization models with consideration of the probabilistic factor. Let us add to our consideration the vector of set of optimizing variables $w = (v, u)$, vector of set of parameters of water quality $y = (y_{\kappa})$ to $\overset{*}{\kappa} \varepsilon \overset{*}{K}$ of control section lines, as well as vector of set of nominal parameters $\alpha \varepsilon Q$, which determines removal of impurities with surface run-off. The same vector may include other nominal parameters: nominal debit of river, self-purification coefficients, etc.

In these terms, the single-step problem of optimizing water-protection measures with given nominal parameters has the following appearance

$$\min_{w \in W} \tilde{f}(w, a) \tag{38}$$

$$W = \{w : s(w, a) = Y,\ Y \in \Omega,\ a \in Q^{q}\} \tag{39}$$

where f is a function defining expenses for water-protection measures and is the set of permissible concentrations of impurities (maximum permissible concentration, MPC), for example, $\Omega = \{y_{\xi} : y_{\xi} \leq MPC_{\xi}\}$.

Let calculation have been made of optimum $\overset{\circ}{w}$ with given nominal values for parameters α. The actual values of α are stochastic, so that they can assume random values of ζ, for which determination can be made of function $\mathcal{Y}(\zeta)$. It is rather obvious that if the nominal and actual values of parameters do not coincide, i.e., $\alpha \neq \zeta$, the actual expenditures $\tilde{f}(\overset{\circ}{w}, \zeta)$ do not coincide with the "nominal optimum" of $\tilde{f}(\overset{\circ}{w}, \alpha)$. This is what leads to some loss in implementing nominally optimum water-protection measures determined by loss function $F(\alpha, \zeta)$.

Construction of function of losses $F(\alpha, \zeta)$ in overt form can ensue from the following considerations. Let the value of ζ be such that $y(\alpha) < y(\zeta) > MPC$,

MPC, when there is infraction of water quality standards and loss could be defined as detriment to water consumers with use of polluted water, in the fish industry and other sectors of the national economy (Anono 1982). On the other hand, these losses could equal losses related to the basic industry, either expenses for compensatory measures (drawdown from reservoirs, additional treatment before using water, and finally, enterprise shut-down).

If the values of ζ are such that $y(\alpha)>y(\zeta)<MPC$, the "nominally optimum" expenditures for water-protection measures $\tilde{f}(\overset{\circ}{w}, \alpha)$ exceed the required minimum to obtain standard water quality $\tilde{f}(\overset{*}{w}, \zeta)$. In this case, the loss $F(\alpha, \zeta)$ equals the difference between "nominal optimum" expenditures and minimum required, i.e., it equals the "superfluous" expenditures.

If $y(\alpha) = y(\zeta)$, which occurs with, for example, $\overset{\circ}{y} = y$, the losses equal zero. Thus, the loss function $F(\alpha, \zeta)$ has a minimum with $\overset{\circ}{y} = y$ and two branches $F_1(\alpha, \zeta)$ and $F_2(\alpha, \zeta)$. . In the above interpretation, the task of optimizing water protection measures with consideration of random factors amounts to the known problem of optimum planning of production under conditions of random output and demand (Volgin 1968, Pervozvanskiy 1975).

The general formulation of the problem can be reduced to the following:

$$\left\{\tilde{F}(a) = \int_{a_{min}}^{a} F_1(a,\zeta)\varphi(\zeta)d\zeta + \int_{a}^{a_{max}} F_2(a,\zeta)\varphi(\zeta)d\zeta\right\} \to \min_{a} \qquad (40)$$

The purpose of this problem is to find a value for nominal parameters α, with which the values of W enable us to obtain minimum overall losses set by functional (40).

Let us note that this solution does not provide as a mandatory situation one in which water quality standards are not met if, in building the loss function, additional expenses for compensatory measures that stabilize the quality of water in "adverse" case, for example, expenses for drawdown from reservoirs, are taken into consideration as losses.

The main problem in practical tasks of optimizing water-protection measures with consideration of random processes is that it is necessary to take into consideration fluctuation of many parameters of the water course. At the same time, the methods of finding the minimum of functional (40) are quite simple if α is scalar and ζ is a one-dimensional random process. Problem (40) can be reduced to a one-dimensional one by standard methods (Korn and Korn 1973).

With consideration of the submitted functions, the order in which the problem is solved is as follows:

- Solution of series of problems of optimization of water-protection measures at minimal overall expenditures, with restrictions on water quality, with outlay in water course within given limits as the variable parameter.

- Determination of function of losses of two types: loss due to inadequate actual level of water-protection measures for the actual discharge of water in the channel [or water course] and loss related to implementation of water-protection measures, the efficacy of which exceeds the minimum required level for actual discharge of water in channel.

- Determination of mathematical expectation of overall losses at given planned (nominal)levels of water discharge in channel, as well as possible variations of water quality.

- Determination of optimum nominal discharge of water in channel in order to plan water-protection measures on the basis of minimizing mathematical expectation of total loss at given densities of probability of random values.

- Determination is made of final solutions to problem of selection of water-protection measures with optimum nominal discharge.

Water discharge in a channel (river) was chosen as the sole nominal parameter for the following reasons. In the first place, such a parameter is the basic factor in forming water quality and it is also a random variable. The methods of building probabilistic characteristics of discharge and run-off have been well-studied. In the second place, surface run-off and river discharge are related by balance functions, so that one can construct the characteristics of a one-dimensional random process determining removal of an impurity with surface run-off and variations in river discharge. For this reason, one can select a certain minimal discharge as α_{min} in (40), and then α is the sought nominal discharge.

SOME PRACTICAL RESULTS AND CONCLUSIONS

Practical studies of the above-discussed models of optimization and forecasting of coordinated development of industry and water-protection complexes remain to be done in order to develpo rather complicated systems of software for computers, as well as a series of digital experiments, including those for actual systems. For this purpose, a problem-oriented package of variable programs (PPP) for forecasting water quality and optimizing waterprotection measures, and some program complexes forming as a whole the OKVOPLAN (optimum integrated water-protection planning and forecasting) were developed at the All-Union Institute of Water Protection.

Calculations on a computer with use of the above-mentioned models yielded the following results:

- Determination was made of MPD that are optimum with respect to minimal expenditures for water-protection measures for several river basins. The "Treatment and utilization" and "Water system" units, and surface runoff in the deterministic variant were taken into consideration. The variants of development of industrial technology were set on the basis of the results of multivariant forecasting (direct reading).

- A study was made of the efficacy of developing intersector water-management systems with optimization of technology for utilization of heat of water from heat and electric power plants (TES) to meet the needs of the fish industry and agriculture. The calculations revealed that there was substantial reduction of expenditures for development of industry and water protection in the intersector complex (TES, hothouse, irrigation system, fishery). The decline of expenses constituted about 100% with 1 MW increment in TES capacity.

- A study was made of the efficacy of introduction of new technologies for steel production with out blast furnace in ferrous metallurgy, and effect on expenditure indicators for the water-protection complex.

- A study was made of probabilistic run-off on choice of optimum water-protection measures, with determination of the need to establish different nominal river discharge, depending on the concrete situation. Standard nominal discharge at the 95% level is usually not the optimum according to the meaning of (40).

On the whole, our studies lead to the conclusion that coordinated development of industrial technology and water-protection technology makes it possible to reach the production goals and those of environmental protection at substantially less cost than if they are developed separately. Furthermore, an optimum combination of rates of development in the "Industry and "Treatment and utilization" units leads to substantial improvement of production efficiency indicators with concurrent solution of problems of water protection.

The difficulty of calculating optimum programs of coordinated development of industrial and water-protection technological complexes makes it necessary to develop special methods to design efficient routines for the computer. One method of constructing such routines is described in the Appendix.

BIBLIOGRAPHY

Anon. 1982. Okhrana okruzhayushchey: modeli sotsial'no-ekonomicheskogo prognoza (Environmental Protection: Models of Socioeconomic Forecasting).

Korn, G. and T. Korn 1973. Spravochnik po matematike dlya nauchnykh rabotnikov i inzhenerov (Guide of Mathematics for Scientific Workers and Engineers). Moscow, USSR. 831.

Pasyuga, N. P. 1985. Hydorlogical support of development of set of water-protection measures in: Regulirovaniye kachestva prirodnykh vod (Control of Quality of Natural Waters). Kharkov, USSR. pp 79-87.

Pervozvanskiy, A. A. 1975. Matematicheskiye modeli v upravlenii proizvodstvom (Mathematical Models in Control of Industry). Moscow, USSR. 616.

Pospelov, G. S. and V. A. Irikov 1976. Programmno-tselevoye planirovaniye i upravleniye. Vvedniye (Special-Purpose programmed Planning and Control. Introduction), Moscow, USSR. 440.

Sukhorukov, G. A. 1977. Planning production and development in hierarchic control systems in: Modeli i metody analiza ekonomicheskikh tselenapravlennykh sistem (Models and Methods of Analysis of Economic Goal-Oriented Systems). Novosibirsk, USSR. pp 58-71.

Vavilin, V. A. and M. Yu. Tsitkin 1977. Mathematical modeling and control of water environment. (5):114-132.

Volgin, L. N. 1968. Problema optimal'nostey v teoreticheskoy kibernetike (Problem of Optimums in Theoretical Cybernetics). Moscow, USSR. 160.

APPENDIX

Current methods of theory of hierarchic systems make it possible to reduce the complicated task of optimizing the program for development of industrial and water-protection complexes to solving a set of simple local problems united by the coordination procedure. The body of this paper described the structure of one of these methods, in which one first solves problem (11-25) for the period [0,T], after which one solves problem (27-37), which has an hierarchic structure for the first stage, t = 1. We propose below a method of hierarchic optimization that permits determination of the program of development for all $t \in T$.

The method is based on aggregation in time of controls of development u(t) in intervals of T, T-1, etc., and analysis of the problem in these intervals with use of aggregation of variables $(V_{i\theta}) = V$ and methods of theory of duality. Let the initial problem of optimization of development program have the following appearance:

$$\left\{ f(u,v) = \sideset{}{'}\sum_{t} C_u' u(t) + c_v' v(T) \right\} \rightarrow \min, \quad t = 1 \dots T,$$
$$Bv(t) = \omega_t, \quad Dv(t) - \sideset{}{'}\sum_{\tau=1}^{t} u(\tau) \leq Z(0,t), \qquad (41)$$
$$Q_p u(t) \leq \beta_t,$$

The aggregated restriction variables have the following appearance:

$$\tilde{u}_T = \sideset{}{'}\sum_{t \in T} u(t), \quad \tilde{u}_{T-1} = \sideset{}{'}\sum_{t \in T-1} u(t), \dots, \quad \tilde{\beta}_T = \sideset{}{'}\sum_{t \in T} \beta_t, \quad \tilde{\beta}_{T-1} = \sideset{}{'}\sum_{t \in T-1} \beta_t$$

Let us substitute the solution to the initial problem (41) with the step-by-step solution of sequence of problems at the following steps:

$$\begin{gathered}\Big\{ \underset{T}{\tilde{f}}(u,v) = c'_u \tilde{u}_T + c'_v v(T) \Big\} \to \min \\ Bv(T) = \omega_T, \quad Dv(T) - \tilde{u}_T \leq Z(0,T), \quad Q_\beta u(T) \leq \tilde{\beta}_T \end{gathered} \tag{42}$$

$$\begin{gathered}\Big\{ \underset{T-1}{\tilde{f}}(u) = c'_u \tilde{u}_{T-1} \Big\} \to \min, \\ Bv(T-1) = \omega_{T-1}, \quad Dv(T-1) - \tilde{u}_{T-1} \leq Z(0,T-1), \\ \tilde{u}_{T-1} + u(T) = \tilde{u}^*_T, \quad Q_\beta \tilde{u}_{T-1} \leq \tilde{\beta}_{T-1}, \quad Q_\beta u_T \leq \beta_T \end{gathered} \tag{43}$$

where $u^*_{\underline{T}}$ is the result of solving the problem at preceding step T. The solution continues analogously (43) to step t = 1.

It was shown that, if solution (41) exists an step-by-step routine has admissible solutions, solutions (42, 43) coincide with the solutions to the initial problem (41).

Let us turn to solving a problem such as (42) or (43) at each step. In scalar form, (42) has the following appearance:

$$\Big\{ f(v,u) = \sum_{i \in I}{}' \sum_{\theta \in \theta_i}{}' \left(c_{vi\theta} v_{i\theta} + c_{u\theta} u_\theta \right) \Big\} \to \min, \tag{44}$$

$$\sum_{\theta \in \theta_i}{}' v_{i\theta} - \sum_{j \neq i}{}' \sum_{\theta \in \theta_j}{}' b_{ij\theta} v_{j\theta} = \omega_i, \tag{45}$$

$$v_{i\theta} \in V_i(u_\theta), \qquad u_\theta \in U_i. \tag{46}$$

Decompositon of (44-46) leads to local problems such as:

$$\Big\{ f_i(v,u) = \sum_{\theta \in \theta_i} \left(c_{vi\theta} v_{i\theta} + c_{u\theta} u_\theta \right) \Big\} \to \min, \tag{47}$$

$$\sum_{\theta \in \theta_i} v_{i\theta} = \mu_i(h-1), \tag{48}$$

$$\sum_{\theta \in \Theta_i}{}' b_{ji\theta} V_{i\theta} \leq r_{ji}(h-1), \tag{49}$$

$$V_{i\theta} \in V_i(u_\theta), \quad u_\theta \in U_i, \tag{50}$$

where μ and r are coordinating parameters and h is the number of iteration with formation of μ and r.

The values of μ and r are calculated using the formulas,

$$\mu_i = \tilde{V}_i, \ r_{ji} = \tilde{b}_{ji} \tilde{V}_i, \ \tilde{V} = (\tilde{V}_i), \ \tilde{V} = (I - \tilde{B})^{-1}, \ \tilde{B} = [\tilde{b}_{ij}],$$

where $\tilde{v}_i$ is a variable aggregated for $\theta \in \theta_i$, $\tilde{B}$ is aggregated square matrix for $\theta \in \theta_i$. The values of aggregated coefficients of matrix $\tilde{B}$ are calculated using the following formulas:

$$\tilde{b}_{ji}(h) = \tilde{b}_{ji}(h-1)\hat{\rho}_i^h + b_{ji\theta}(1 - \hat{\rho}_i^h), \quad i = i^*, \ \theta = \theta^*$$

$$\tilde{b}_{ji}(h) = \tilde{b}_{ji}(h-1), \quad i \neq i^*,$$

where $\hat{\ }^h$ is length of step at hth iteration, which is determined by the "golden section" method; $\ \varepsilon [0,1]$, i* is the number of the "leading" subsystem in the hth interation, θ^* is the number of the "leading" column, which is identified with the formula,

$$\Delta^*_{i^*,\theta^*} = \min_{i \in I} \{ \Delta_{i\theta^*} \}$$

$$\Delta_{i\theta^*} = \min_{\theta \in \Theta_i} \left\{ \tilde{V}_i(h-1) \left(\sum_{j \neq i}{}' [(\overset{*}{\lambda}_j - \overset{*}{\gamma}_{ji})(b_{ji\theta} - \tilde{b}_{ji}(h-1))] \right) \right\} \tag{51}$$

where $\lambda^*_j \ \overset{*}{\gamma}_{ji}$ are dual evaluations of (48, 49).

Some details referable to logic of choosing i*, θ^* were omitted in formula (51). It was shown in practice that iterative solution of (47-51) amounts to solution of (44-46).

GREAT LAKES AGRICULTURAL POLLUTION CONTROL IN PERSPECTIVE

by

V.J. Saulys[1]

ABSTRACT

Major efforts are underway in the Great Lakes area to control environmental pollution, particularly with respect to nonpoint agricultural sources. Farmers in the Lake Erie Basin have joined with environmental and soil conservation experts to institute programs to control these sources. Their work has demonstrated the potential of no-till or reduced tillage practices to reduce phosphorus loadings to streams and lakes. The potential for increased pesticide pollution is of growing concern, however.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

The Great Lakes, the "sweet water seas" of North America, have long served to focus United States' energies on pollution control. President Lyndon Johnson, when urging the Congress to pass the Federal Water Pollution Control Act of 1965, said that Lake Erie, for all intents and purposes, was "dead." Some years later, Mr. William Ruckelshaus, the first Administrator of the U.S. Environmental Protection Agency (EPA), noted that he once met with officials from the City of Cleveland (Ohio). They assured him that the pollution problems of the Cuyahoga River, a major tributary of Lake Erie, had been solved. "Next week," Ruckelshaus said, "the river caught fire and burned down two bridges and a houseboat" (Lake and Morrison 1977a). Such incidents, together with beaches closed by raw sewage and open beaches where rotting mats of algae and floating rows of dead fish competed with people for sand and water, stirred the people and their elected officials to action.

---

[1] U.S. Environmental Protection Agency, Region V, Great Lakes National Program Office, Chicago, IL 60605 USA

The pollution sources in the Great Lakes Basin were quickly identified and targeted. These sources could be grouped into classes and categories (Table 1).

TABLE 1. POLLUTION SOURCES

	Location	
Source	Urban	Rural
Point	Municipal discharges Industrial discharges Atmospheric releases	Human waste systems Animal feed lots Agricultural chemical and waste spills
Nonpoint	Hazardous waste sites Municipal waste landfills Stormwater runoff	Erosion Improper land use Pesticide misuse Fertilizer management

A decade ago the problems were highly visible and priorities apparently simple. The point sources of pollution, particularly, industrial, were highly visible. Thus, they were the first targets.

Today, many of these pollution sources are under control or have control programs to address them. Municipal and industrial discharges must meet strict federal technology-based requirements and state water quality standards. Phosphorus controls were imposed on sewage treatment plants and large industrial sources in the Great Lakes Basin to slow the rate of eutrophication. As a result, the phosphorus loadings to Lake Erie from these sources decreased from 15,260 metric tons in 1972 to 2,541 metric tons in 1982 (Figure 1).

Similar progress was reported for the industrial section. For example, the U.S. paper and pulp industry, once one of the "dirtiest of polluters," is now one of the exemplary industries (Anon. 1981) (Figure 2).

Less progress has been achieved with urban nonpoint runoff. Many large cities with serious problems in this area in the Great Lakes Basin, however, have programs to capture and treat storm runoff or separate storm runoff from domestic waste. The $3.2 billion surface and subsurface capture programs in Chicago, Illinois, the $1.6 billion project in Milwaukee, Wisconsin, and smaller projects in Rochester, New York, and elsewhere are examples of such programs. Many open municipal and industrial dumps were buried during the 1960s and early 1970s to eliminate surface water pollution. These programs turned out to be, in many instances, an example of sweeping a problem

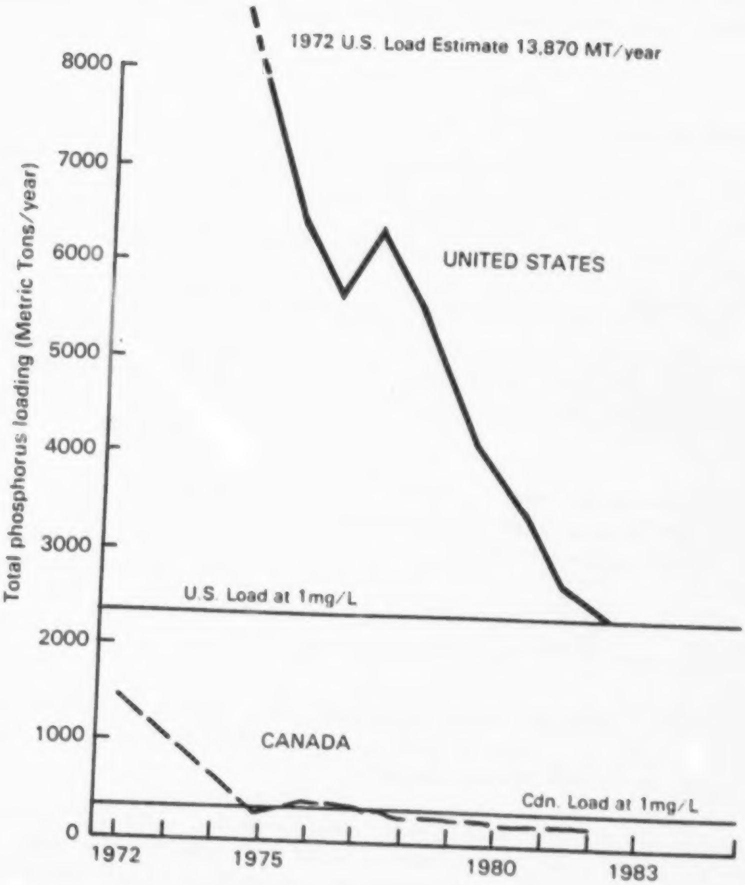


Figure 1. Lake Erie municipal phosphorus loads

under a carpet. Today, a massive state and federal effort is underway to correct the resultant groundwater and surface water pollution created by past expedient actions.

Major efforts are underway to address large atmospheric sources of contaminants. While much of the public's attention is on "acid rain," U.S. and European research studies are gathering evidence that the rain may not be only an acidic, but also a toxic, cocktail. Although much remains to be done in the atmospheric control area, the basic technology to reduce sulphur and nitrogen oxides has been identified. Many necessary controls have been put in place on mobile (vehicles) and stationary sources of pollution. As of April 1983, 106 industrial facilities and electric power plants in the United States have sulphur dioxide control technology in place. An additional 35 have sulphur dioxide scrubbers under construction; and 70 more facilities are under consideration for sulphur removal technology (Personal communication, EPA Region V, Chicago, IL. July 1984).

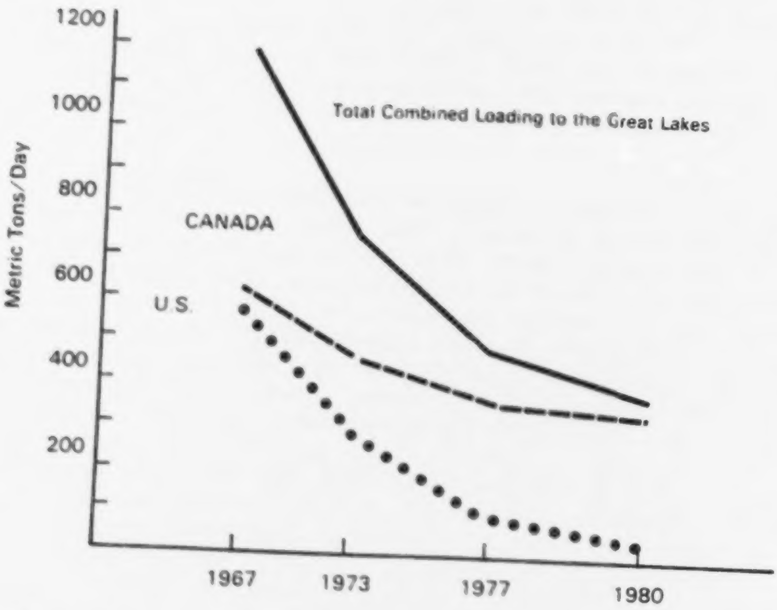


Figure 2. BOD_5 Discharge trends of paper and pulp mills discharging directly into the Great Lakes

Much progress has been achieved in cleaning up industrial and municipal sources. Beaches closed for decades and ignored have been reopened as health warning signs are removed one by one. Lake Erie, once pronounced dead, now supports a renewed walleye sport fishery bringing $100 million per year to the economy of the State of Ohio alone. The Cuyahoga River shoreline, once a fire hazard, is now the site of new restaurants and luxury condominiums.

Rural point sources also have seen progress in pollution control. Regulations have been passed to eliminate large feedlot runoff, to prevent pesticide misuse, and to contain agricultural chemical and waste spills. Violators face large monetary fines. Small rural communities are encouraged to install water treatment systems designed for low capital costs and low maintenance. Monetary assistance is available from state and federal agencies for design and installation of municipal treatment systems. Regulations have been tightened by local government bodies to ensure the proper design, location and installation of individual household treatment systems. The engineering solutions, for the most part, have been identified. Their implementation, in many instances, is nearly complete.

The major remaining pollutant impact upon the Great Lakes ecosystem comes not from point sources but from nonpoint agricultural sources. It has been the farmer -- not the city dweller or the industrial baron -- who has shaped the Great Lakes ecosystem. True, the cities have done their share to damage the ecosystem, and, industry has contributed much more than its share of pollutants, particularly those that are toxic. The farmer, by changing the face of the land, changed the ecology of the Great Lakes forever. Unless agricultural nonpoint sources of pollution are controlled, however, the investment made in cleaning up urban sources of pollution will not produce the full benefits. We have gone past the point where only the engineers will provide the answers. We have reached the point of resource management. Today, the agronomists and the ecologists will have to provide the leadership to complete the cleanup of the Great Lakes and to ensure their proper management.

This situation was foreseen on January 7, 1972, during a conference on Maumee River pollution problems. The participants of this conference were told by the EPA representative that the Maumee River was the major contributor of silt and associated nutrients to Lake Erie. The participants were advised that the initiatives necessary to control industrial and municipal pollution were underway. The EPA representative also forecast that "when these problems are solved, the problems of agricultural pollution will remain" (Lake and morrison 1977a). Among those conference participants were farmers from the Allen County (Indiana) Soil and Waste Conservation District. They did not like what they heard and resolved to do something to change the prophecy. They held a series of meetings involving agricultural and environmental agencies at the local, state, and federal levels. Their timing was right and, within a few months, agreement was reached to undertake a research program involving agricultural and environmental specialists to answer three questions (Lake and Morrison 1977c).

> Could a conventional program of land treatment, undertaken in the Maumee Basin, result in a measurable improvement in the water quality in Lake Erie?
>
> What would the cost be of such a project and could the dollars spent be correlated with an improvement in water quality?
>
> What programs, incentives, and administrative techniques would result in the most nearly complete participation by landowners?

The research program was to take place in Allen County with the full support of the Allen County Soil and Conservation District. Thus, the Black Creek Project was born in 1972. It was the first detailed look at the contributions of agriculture to the degradation of water quality in the United States.

The Black Creek watershed was chosen because it was representative of the Maumee Basin's land use and soil types (Table 2). It was large enough (4872 hectares) to allow for sufficient studies of the sub-watershed and the differing land uses. The Maumee Basin extends into the states of Ohio, Indiana and Michigan. It is one of the last of the areas in the Lake Erie Basin to be settled (mid-19th century) because it was the site of the "Great Black Swamp," the remains of the glacial Lake Maumee. Until the arrival of German settlers with experience in farming heavy clay soils, it was not considered fit for serious agriculture. Today, it is one of the most productive agricultural areas of the United States.

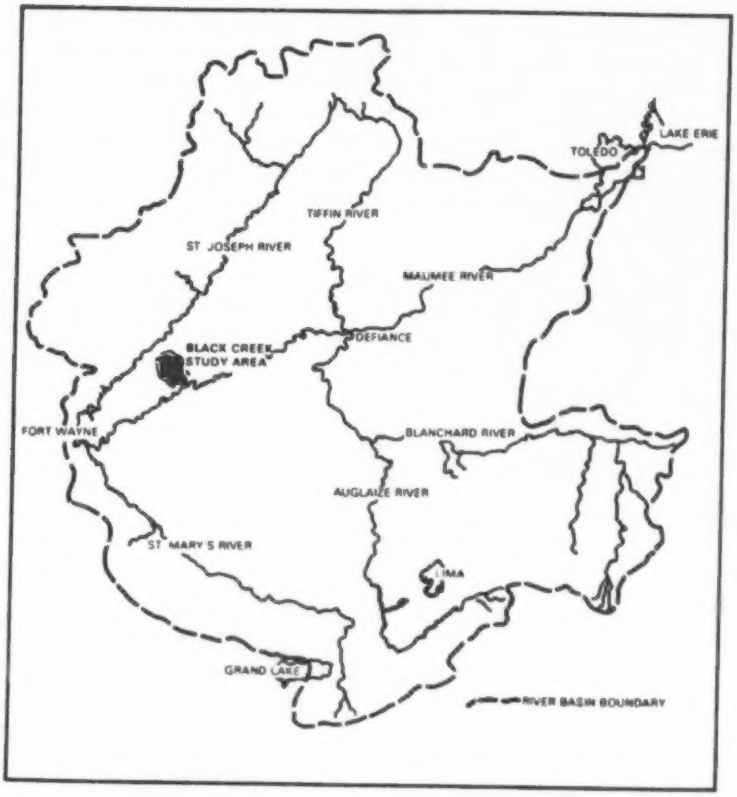

LAKE ERIE
TOLEDO
TIFFIN RIVER
ST JOSEPH RIVER
MAUMEE RIVER
DEFIANCE
BLACK CREEK STUDY AREA
FORT WAYNE
BLANCHARD RIVER
AUGLAIZE RIVER
ST MARY'S RIVER
LIMA
GRAND LAKE
RIVER BASIN BOUNDARY

Figure 3. Maumee Basin--Black Creek Location

TABLE 2. BASIN AND SUB-BASIN LAND USE*

Maumee River Basin	Black Creek Basin
1,711,493 hectares	4,872 hectares
73% cropland	80% cropland
4% pasture	4% pasture
80% woodland	4% woodland
9% urban	9% urban

*Lake and Morrison (1977a).

The Black Creek study consisted of a research program to define the water quality and biological parameters of Black Creek and an administrative program to test the pollution control benefits of known soil conservation technologies. During a 5-year study period, it was found that the traditional grab sampling methods were inadequate to accurately show the true load of sediments and related pollutants discharged by Black Creek to the Maumee River. Automated samplers had to be installed at several sampling locations. This equipment allowed the scientists to collect more frequent and, thus more accurate, information on water quality and sediment load following a rainfall event. The information provided by these samplers showed that a single rainfall event could deliver as much as 86% of the total annual sediment load.

The Black Creek chemical parameters were typical of such agricultural watersheds: nitrate-nitrogen runoff of 2 to 20 kg/ha, phosphorus levels significantly above Lake Erie objectives (0.05 - 0.16 mg/l versus 0.01 mg/l target), and an aquatic community dominated by pollutant-tolerant species (Lake and Morrison 1977a).

The agricultural portion of this study planned to investiage the cost and benefits of 30 agricultural soil control practices and combinations. These were grouped into three categories:

Structural practices

Diversion structures to reduce length of slope
Slope stabilization structures
Grassed waterways to convey storm water to streams
Holding ponds and tanks for animal wastes
Land smoothing
Fences to keep livestock away from streams
Special livestock watering facilities
Ponds
Sediment control basins
Stream channel stabilizers
Stream bank protectors
Surface drains
Gradient terraces
Paralleled terraces
Tile drains

Cultivation practices

Contour planting
Crop residue management
Minimum tillage
Pasture and hayland planting
Strip cropping
Improved harvesting of woodlands
Woodland improvement
Woodland pruning

Combined structural and cultivation practices

- Farmstead and feedlot windbreaks
- Field border plantings (hedgerows, etc.)
- Field windbreaks
- Reforestation
- Sediment control during construction
- Recreation area improvements via plantings
- Wildlife habitat management

The study yielded answers in a number of areas. Of the above listed structural and cultural practices, nine were chosen as constituting Best Management Practices for the Black Creek:

- Field borders
- Grade stabilization structures
- Grassed waterways
- Fences for livestock
- Pasture planting
- Sediment control basins
- Terraces
- Limited channel protection
- Tillage methods that increase crop residue and surface roughness.

A mathematical model called ANSWERS (Aerial Nonpoint Source Watershed Evaluation Simulator) was developed as part of the project. It was shown to predict the effect of an actual storm event loading within 15% of that measured in Black Creek. The model also was used to identify the most critical areas within the watershed and simulate the effect of changing from conventional tillage (using the molboard plow) to minimum tillage (using the chisel plow). This simulation showed that 40% of the reduction in soil loss could have been achieved by treating 32 hectares in the most "critical" areas rather than the 728 hectares affected by the project (Lake and Morrison 1977a).

Work with rainfall simultors on small plots confirmed that crop residues and tillage practices, which prevent soil mobilization, were more significant in preventing soil loss than those practices aimed at intercepting mobilized soil. Once a rain drop's impact mobilized the soil particles, it was an uphill struggle to keep the soil from the stream.

Furthermore, the biological studies showed that several of the structural changes, insisted upon by the local farmers, such as stream channelization and stream bank protection, did little to reduce total loss of soil but significantly damaged the stream's biological community (Lake and Morrison 1977b).

At the conclusion of the study, the U.S. Army Corps of Engineers, which had been required by the U.S. Congress to "develop a demonstration wastewater program for the rehabilitation and environmental repair of Lake Erie," took the findings of the Black Creek project and applied them to a nearby watershed in Ohio. The Honey Creek Project focused on no-till and minimum tillage practices. As seen in Table 3, the project confirmed that they were the cheapest and most effective measures to reduce phosphorus and to rehabilitate Lake Erie.

The Maumee River and other Lake Erie tributaries are estimated to deliver an average of 8400 metric tons of agricultural nonpoint phosphorus per year to Lake Erie (U.S. Army, COE 1983). Since in 1982 the total phosphorus input to Lake Erie was estimated at 12,349 metric tons, a reduction in agri-

TABLE 3. COSTS OF METHODS FOR REDUCING PHOSPHORUS RUNOFF*

Method	Total phosphorus $/kg	Biologically available phosphorus, %	$/kg of Biologically available phosphorus
Rural nonpoint sources			
No-till and conservation tillage	0	25	0
Cover crops	276.00	25	1,100.00
Critical area seeding	326.00	25	1,300.00
Contour stripcropping	82.00	25	330.00
Diversions	2,640.00	25	10,600.00
Waterways	97.00	25	390.00
Vegetative filters	30.00	25	120.00
Runoff control structures	368.00	25	1,470.00
Terraces	73.00	25	290.00
Tile drains	9,180.00	25	36,720.00
Manure storage and spreading	4.40	75	5.90-36.3
Barnyard runoff control	2.20	75	2.90
Fertility management	0	25	0
Fertilizer placement	44.00	25	176.00

*U.S. Army, COE (1983).

cultural contribution is necessary to meet the 11,000 metric ton phosphorus loading target set by the 1978 Great Lakes Water Quality Agreement between the United States and Canada (Great Lakes Water Quality Board 1983). The results of the Lake Erie Wastewater Management Study indicates that, with use of no-till and reduced tillage practices, the achievement of this target is feasible (Table 4).

TABLE 4. EXISTING AND POTENTIAL ACHIEVABLE REDUCTION OF SOIL EROSION IN THE UNITED STATES LAKE ERIE DRAINAGE BASIN* (million metric tons per year)

Conservation practices	Western basin	Central basin	Eastern basin	Total Lake Erie basin
Base year (1975)	16.6	6.7	1.8	25.1
Reduced tillage only (percent reduction)	8.3 (50)	3.9 (42)	1.3 (28)	13.5 (46)
No-till and reduced tillage (percent reduction)	4.5 (73)	2.4 (64)	0.9 (50)	7.8 (69)

*U.S. Army, COE (1983).

In late 1980, the information available from the Lake Erie Wastewater Management Study and the reanalysis of the Black Creek data using the ANSWERS model made it evident that the no-till and minimum tillage practices would significantly reduce the amount of phosphorus reaching Lake Erie. The information indicated that there would be little, if any, additional cost to the farmer, if he was trained in the use of new equipment and practices. Therefore, EPA Region V, U.S. Army Corps of Engineers, and Department of Agriculture representatives signed an agreement in 1981 to jointly work toward the acceptance of these practices. It was agreed that the most effective way to introduce a change in farming practices would be to fund the local soil conservation districts to demonstrate the use of the new techniques and equipment by trained local members of the farming community.

This proposal for conservation tillage demonstration funding was enthusiastically received by the local soil conservation districts. Projects have been funded in 31 counties in the Lake Erie Basin. The projects targeted "critical areas" where erosion would result in the greatest phosphorus control. During 1983, the projects tracked the performance of 1854 demonstration farm plots covering a total of 9472 hectares. These plots were analyzed for costs of production (including fertilizer, pesticide, and machinery use), crop yields, and estimated soil loss. The preliminary conclusions from this demonstration program indicate that:

1. Yields with notill and ridgetill were competitive with yields produced under conventional tillage systems.

2. Costs of production for conservation tillage systems were less than or equal to costs of producing the same crops using conventional systems.

3. Conservation tillage systems reduced phosphorus loadings from the project area and did not significantly increase herbicide use (4 to 12%).

Although only 9400 hectares are being closely monitored by the program, about 22% of the 3,237,500 hectares of farmland was in some form of conservation tillage in 1983. The demonstration projects should significantly increase this percentage in the coming years (U.S. Environmental Protection Agency 1984).

One of the principal concerns about encouraging the farmers to switch to no-till or reduced tillage farming practices is the potential for increased use of pesticides. There are two conflicting schools of thought on the subject:

1. The chemical usage will increase because the conservation tillage practices may rely upon pesticides to control weeds and insects.

2. The farmers currently make heavy use of these chemicals, thus, conversion to conservation tillage practices with close attention to chemical use should result in similar or lower pesticide usage.

The existing literature reveals little information on current pesticide usage in the Lake Erie Basin. The information, which is currently available, is significantly out of date (1978 and earlier). Recent work (Baker, 1983) shows that the amount of pesticides present in runoff from Lake Erie agricultural areas can be significant.

These pesticide loadings can be contrasted with the organic contaminants discharged by industrial and municipal sources to the Niagara River, which drains Lake Erie into Lake Ontario (Table 6).

The latter loadings are deemed to be of enough significance as to call for special cooperative measures between U.S. and Canadian pollution control agencies to identify and reduce the sources. Granted, the formulations of the pesticides measured in Maumee and other rivers are such that they should degrade readily in the environment. However, recent findings by Baker (1983) of concentrations of atrazine, alachlor, and metolachlor in public water supplies similar to the stream concentrations indicate that the degradation mechanisms are not as quick as they were assumed to be. Sampling of Lake Erie waters has not indicated a build-up to date of these compounds. No attempt has been made, however, to assess the impact of pulsed loadings to sensitive fishery areas during the annual reproductive cycle. Nor have these compounds been assessed as to their synergistic, lifetime exposure impacts on human health.

TABLE 5. ESTIMATED PESTICIDE LOADS (kg) FOR THE PERIOD BETWEEN MAY 1, 1982, AND JULY 31, 1982*

	Total loading		
Pesticide	Honey Creek	Sand. River	Maumee River
Metolachlor	241	1750	2920
Atrazine	223	1600	4240
Alachlor	89.2	1290	2820
Metribuzin	27.5	518	1370
Cyanazine	24.7	226	1590
Simazine	19.7	179	1280
Linuron	62.4	264	571
DEA	29.4	168	490
DIA	52.0	130	629
Chlorpyrifos	9.47	135	226
Penoxalin	2.38	19.3	95.4
Ethoprop	1.06	3.86	34.7
Totals	781.8	6,283.2	16,266.1

*Baker (1983).

TABLE 6. CONTAMINANT GROUPS DISCHARGED INTO THE NIAGARA RIVER*

Contaminant group	Total loading to river, kg/day (lb/day)
Acid extractables	46 (100)
Polynuclear aromatics	17 (38)
PCBs	0.11 (0.24)
Pesticides	1.6 (3.5)
Other base/neutral extractables	30 (66)
Purgeables	140 (310)

*Anon. (1984).

In summary, the Great Lakes have long served as an early warning system for the world about environmental problems. They also have served to demonstrate that close international cooperation can reverse trends in existence for decades and even solve environmental problems. The control of phosphorus and eutrophication in Lake Erie is an excellent example.

The Lakes have shown us clearly that soil and its attendant chemicals is a major pollutant, destroying the ecological balance of one of the streams and aging the Lakes. The consequences of land runoff control strategies are only now being assessed. The new challenge is to keep the soil on the farm as a resource for future generations while feeding the increasing population of the world.

REFERENCES

Anon. 1981. The response of the pulp and paper industry in the Great Lakes Basin to pollution abatement programs. Report to the Great Lakes Water Quality Board: Pulp and Paper Point Sources Task Force of the Water Quality Programs Committee. 68 pp.

Anon. 1984. Niagara River Toxics Committee Draft Report. Unpublished.

Baker, D.B. 1983. Studies of sediment, nutrient and pesticide loading in selected Lake Erie and Lake Ontario tributaries - draft final report. Unpublished.

Great Lakes Water Quality Board. 1983. 1983 report on Great Lakes Water quality. Great Lakes Water Quality Board Report to the International Joint Commission. 97 pp.

Lake, J., and J. Morrison. 1977a. Environmental impact of land use on water quality: Final report on the Black Creek Project - Summary. Great Lakes National Program Office, Chicago, Illinois. EPA-905/9-77-007-A, United States Environmental Protection Agency. 94 pp.

Lake, J., and J. Morrison. 1977b. Environmental impact of land use on water quality: Final report on the Black Creek Project - Supplemental Comments Great Lakes National Program Office, Chicago, Illinois EPA-905/9-77-007-D, United States Environmental Protection Agency. 107 pp.

Lake, J., and J. Morrison. 1977c. Environmental impact of land use on water quality - Executive Summary. 12 pp.

U.S. Army, Corps of Engineers. 1983. Summary report of the Lake Erie wastewater management study. U.S. Army, Corps of Engineers, Buffalo District. 31 pp.

U.S. Environmental Protection Agency. 1984. Lake Erie Demonstration Projects: Evaluating impacts of conservation tillage on cost, yield and environment. U.S. EPA, Great Lakes National Program Office, Chicago, Illinois. 17 pp.

MODELING THE FORMATION OF WATER QUALITY IN WATER CHANNELS RECEIVING SURFACE RUN-OFF FROM FARM LAND

by

Ye. V. Yeremenko, V. Z. Kolpak and N. I. Selyu[1]

ABSTRACT

A multicomponent mathematical model is examined for the forming of water quality in a river considering surface run-off from farmlands entering the water course in the form of a continuous-in-length lateral influx of water and matter formed in the watershed. In using the equations for the balance of matter, a system of linear equations has been derived for a mathematical model of transforming the nitrogen compounds and the content of dissolved oxygen in the water. Proceeding from the supposition that low lateral influx does not substantially change the amount of the average rate of the water course and using coefficients of nonconservativeness within the limits of the sections into which the water course is divided, a solution has been obtained for the model's equations in an analytical form that can be used in optimizing water conservation measures.

The necessary information on the amount of lateral influx and the concentration of the designated matter in the surface run-off has been obtained on the basis of the elaborated mathematical model for the water quality of surface run-off. Here the watershed is represented in the form of a systematized aggregate of flat slopes.

Also discussed are the questions of determining the calculation conditions and requirements that should be satisfied by the surface run-off models. As an example, results are given for calculating water quality in the water course considering the lateral influx from the watershed.

INTRODUCTION AND DISCUSSION

Control of water quality in river basins and related setting of maximum permissible run-off (MPR) of drainage water depend largely on conditions of

---

[1] All-Union Scientific Research Institute of Water Protection, USSR Ministry of Water Management, Kharkov, USSR

surface drainage and levels of substances in it for which standards are set. In the most general case, there is migration into water channels of surface run-off from farmland that occupies large territories, in the form of a continuous line of influent water and mass of substances for which standards are to be set.

Evaluation of organized dumping of drainage water and level of surface run-off on water quality in a river can be made by means of appropriate models of water quality. One of these numerous models is described by Yeremenko et al. (1981). We shall discuss a multicomponent mathematical model of formation of water quality, which takes into consideration the role of surface run-off and is aimed at solving water-protection problems. It permits determination of transformation of substances to be standardized and development of elements of a transitional matrix to solve optimization problems of controlling the quality of surface waters. In this model, the substances to be standardized are the basic forms of nitrogen.

Without imposing restrictions on the law of change in water discharge Q in a stream (a linear law of change in water discharge is discussed by Aytsom et al. 1968) and on concentration of substances in surface water N^P, it is considered by Koval'cyuk et al. (1979) that N^P corresponds to background concentration in the stream and using the equation of balance of matter in the section x, x+Δx, we shall have:

$$NQ + N^P \Delta Q = N(x+\Delta x)\, Q(x+\Delta x),$$

which, considering expansions to values on the order of $O(\Delta x^2)$ and $\Delta x \to 0$, leads us to the equation:

$$U\frac{dN}{dx} = -\frac{U}{Q}\frac{dQ}{dx}\left(N-N^P\right) \qquad (1)$$

This equation is the simplest one for consideration of the effect of surface run-off on concentration of matter N. Its right part characterizes a continuous positive ($N<N^P$) or negative ($N>N^P$) source of matter coming in with surface run-off. Considering the nonconservative nature of matter, the initial equation is presented as follows:

$$U\frac{dN}{dx} = -KN - \frac{U}{Q}\frac{dQ}{dx}\left(N-N^P\right), \qquad (2)$$

which yields the following system, as applied to the successive transformation of nitrogen compounds within a section of the stream.

$$
\begin{aligned}
&U\frac{dN_1}{dx}+K_1N_1+\frac{U}{Q}\,\frac{dQ}{dx}N_1=\frac{U}{Q}\,\frac{dQ}{dx}N_1^{p}\\
&U\frac{dN_2}{dx}+K_2N_2+\frac{U}{Q}\,\frac{dQ}{dx}N_2=\frac{U}{Q}\,\frac{dQ}{dx}N_2^{p}+\delta K_1N_1\\
&U\frac{dN_3}{dx}+K_3N_3+\frac{U}{Q}\,\frac{dQ}{dx}N_3=\frac{U}{Q}\,\frac{dQ}{dx}N_3^{p}+K_2N_2\\
&U\frac{dN_4}{dx}+K_4N_4+\frac{U}{Q}\,\frac{dQ}{dx}N_4=\frac{U}{Q}\,\frac{dQ}{dx}N_4^{p}+K_3N_3 \qquad (3)\\
&U\frac{dS}{dx}-K_5(S_K-S)+\frac{U}{Q}\,\frac{dQ}{dx}S=\frac{U}{Q}\,\frac{dQ}{dx}S^{p}-\\
&-\gamma_1K_1N_1-\gamma_2K_2N_2-\gamma_3K_3N_3,
\end{aligned}
$$

where N_1, N_2, N_3, N_4 and S are concentrations of organic, ammonia, nitrite, nitrate nitrogen and dissolved oxygen, δ, γ_1, γ_2 and γ_3 are stoichiometric coefficients, K_1, K_2, K_3, and K_4 are coefficients of nonconservation.

On the assumption that lateral inflow does not alter appreciably the mean flow rate and that the coefficients of nonconservation remain constant within a section, the solution of system 3 can be expressed as follows.

$$
\begin{aligned}
N_1&=G_{11}N_{01}+G_1\\
N_2&=G_{22}N_{02}+G_{21}N_{01}+G_2\\
N_3&=G_{33}N_{03}+G_{32}N_{02}+G_{31}N_{01}+G_3 \qquad (4)\\
N_4&=G_{44}N_{04}+G_{43}N_{03}+G_{42}N_{02}+G_{41}N_{01}+G_4\\
S&=G_{SS}S_0+G_{S3}N_{03}+G_{S2}N_{02}+G_{S1}N_{01}+G_S.
\end{aligned}
$$

where 0 shows values of concentrations of substances at the beginning of the section, Gij and Gsj are elements of the transitional matrix, values of which are listed in Appendix 1.

To determine concentrations of Ni and S, we need information about lateral inflow Qp(x) and concentration of substances NP, which is provided by the model of surface run-off.

Surface run-off is formed in the water catchment area as a result of rain and it usually contains substances in both dissolved form with liquid run-off and on particles of washed away soil, with solid discharge. Thus, the model of surface run-off should provide information about both lateral inflow of water and levels in it of soluble agents, as well as agents carried in with solid discharge.

At the present time, there are methods in the USSR for calculating maximum discharge, rain flood (in water catchments less than 50 km^2 in size) and soil wash-off (Anon. 1975, Anon. 1979). Maximum discharge of rain floods is calculated for water catchment areas up to 50 km^2 in size, while determination of soil wash-off with rain run-off from slopes is made on areas up to 2 km^2 in size (Anon. 1979). The restriction on water catchment area makes it necessary to develop an approach without such a restriction, and to view a water catchment area of arbitrary size in the form of a certain set of slopes in order to be able to use system 3 in estimating soil wash-off. Hence, one of the requirements for a model of formation of surface run-off in a water catchment area is to take into consideration the structure of the catchment area, gully and ravine network over which the surface run-off formed on slopes travels without run-off.

As a rule, precipitation falling in a catchment area has spatial and time variability. For this reason, the second requirement imposed on the model is that it must take into consideration the spatial and time variability of precipitation (as a special case, they could be stationary).

Finally, application of fertilizers in soil is also performed repeatedly on farm fields. Hence the third requirement of models, that of consideration of spatial heterogeneity of distribution of fertilizers in the water catchment area. It should be noted that the requirements imposed on a model of formation of rain-caused flood regions and removal of suspended drift from water catchment areas do not impose restrictions on applicability of the model for the case where these characteristics are constant in time and space.

If these requirements are met, it will be possible to get an answer to the questions of how and where torrential rain water and solid run-off enter the water system (distributed in length or concentrated) when making estimates of formation of run-off in a water catchment area.

By calculating the quality of surface waters formed on slopes and the gully-ravine network, one can learn how the concentrations of substances will change in the run-off as a function of meteorological and agrotechnical factors, as well as environmental protection measures. The latter is particularly important in our opinion: to assess the efficacy of different environmental protection measures involving the entire water catchment area or some segment of it according to volume of substances carried out.

The model of surface run-off consists of several submodels. Let us discuss them.

SUBMODEL OF RUNOFF FORMATION

It is known that the equation of Nash (1957), equation for kinematic wave (Kuchment et al. 1983) and equations of Saint Venant can be used to describe flow of water down a slope (thalweg). In our opinion, Nash's equation is the most suitable for solving practical problems (ordinary first-order differential equation):

$$K \frac{dQ^p}{dt} = J - Q^p$$

where Q^p is efflux of water from slope, I is influx of water to slope (including intensity of precipitations) and K is a coefficient.

However, the equation includes coefficient K, which is difficult to determine, and although we do know from literature sources its expression by hydrolic parameters and slope characteristics, its use with reference to specific water catchment areas requires special validation. For this reason, for a model of movement of torrential rain water on a slope, we obtained an ordinary first-order differential equation that contains no uncertain parameters. It is based on the equation of continuity with consideration of lateral influx:

$$\frac{\partial \omega}{\partial t} + \frac{\partial Q^p}{\partial x} = \frac{(J - r)}{6 \cdot 10^4} B(x, \omega) + q_{\text{бок}}(x, t), \qquad (6)$$

where w is flow cross section, m^2, Q^p is water discharge, m^3/s, I(t) and r(t) are intensity of rain and infiltration, mm/min, B is width of free surface of flow, m, $q_{\delta o \kappa}$ is lateral influx per unit current length (slope or stream), m^2/s, x is a coordinate read downstream, m, t is time, s.

By integrating equation 6 for stream length x from 0 to ℓc we obtain.

$$\frac{d\omega}{dt} = \frac{(J - r)B}{6 \cdot 10^4} + \frac{(q_{\text{вх}} - Q_c^p)}{\ell_c}, \qquad (7)$$

where

$$q_{\text{вх}} = q_0 + \int_0^{\ell_c} q_{\text{бок}}(x, t)\, dx = q_0 + \ell_c \bar{q}_{\text{бок}};$$

q_0 is water discharge in first section of stream with $x = 0$, m³/s, $Q^P{}_b$ is water discharge in last section of stream with $x = c$, m³/s,

$$\bar{\omega}(t) = \frac{1}{\ell_c}\int_0^{\ell_c} \omega \, dx \, ; \qquad \bar{B} = \frac{1}{\ell_c}\int_0^{\ell_c} B \, dx \, ;$$

w is cross section of stream.

Since equation 7 has two unknowns, $Q^P b$ and $w(t)$, let us express $Q^P{}_b$ as $w(t)$, using the formulas of Chezy and Manning, analogously to derivation of the kinematic wave equation from the equation of continuity:

$$Q^P_B = \frac{\sqrt{i}}{n}\,\omega \,\bar{R}^{2/3}(\bar{\omega}), \qquad (8)$$

where $\bar{R}(\bar{w})$ is hydraulic radius averaged for length of current, i is mean slope of current and n is roughness coefficient.

Substituting equation 8 in equation 7, we obtain an ordinary nonlinear first-order differential equation with reference to w (the line over the w was omitted for the sake of simplicity).

$$\frac{d\omega}{dt} = \frac{(J - r)\,B(\omega)}{6\cdot 10^4} + \frac{q_{6x}}{\ell_c} - \frac{\sqrt{i}}{n\,\ell_c}\,\omega R^{2/3}(\omega), \qquad (9)$$

The following initial condition is added to this equation:

$$\omega(0) = 0 \qquad (10)$$

Equation 9 is solved mathematically using well-known methods. As a result of digital integration, we obtain discrete values for current cross section $w_j = jT$, $j = 1, 2 \ldots$, T is the time gradation of integration. The value of discharge is calculated using formula 8 with w_j. The integration step T [gradation] in equation 9 is chosen from the condition of stability of the computing process.

$$\tau \le 2.78 \left| \frac{(J - r)}{6\cdot 10^4}\,\frac{dB(\omega)}{d\omega} - \alpha\,\frac{d\Psi(\omega)}{d\omega} \right|^{-1} \qquad (11)$$

where

$$\alpha = \sqrt{i}\,/\,n\ell_c, \quad \Psi(\omega) = \omega R^{2/3}(\omega).$$

In the case of level run-off from a slope,

using the maximum (stationary) value for w, on the basis of established water run-off.

$$\omega = \omega_{CT} = \left[\frac{(J - r)\, B^{5/3}}{6 \cdot 10^4 \alpha} \right]^{3/5} \quad \text{with} \quad J(t) - r(t) = (J - r)_{max} = Const.$$

we obtain from equation 11:

$$\tau \leq 2.78 \left[\alpha^{3/5} \left(\frac{J - r}{6 \cdot 10^4} \right)^{2/5} \cdot \frac{5}{3} \right]^{-1} \qquad (12)$$

Thus, by integration of equation 9 one can obtain a hydrograph of run-off from a slope. For the calculations i, n, qbx, l, r and ℓc must be known.

SUBMODEL OF WATER QUALITY

Determination of volume (hydrograph) of solid run-off, which is determined entirely by liquid run-off which, in turn, carries off dissolved substances, is an important element in a model for quality of water of surface run-off.

In the model developed at the All-Union Scientific Research Institute of Water Protection for formation of water quality of surface run-off, use is made of the basic thesis in the literature (Anon. 1979). Suspended sediment is estimated using the formula,

$$C_{CH} = 10^{8} \frac{M_{CH} F_{б}}{W_c}, \qquad (13)$$

where M_{ch} is the modulus of run-off of sediments with P% coverage, tons/ha, F_b is size of water catchment areas, km^2, W_c is volume of rain run-off, m^3, C_{ch} is concentration of suspended sediment in run-off, g/m^3. Parameters Wc and M_{ch} are expressed as follows.

$$\begin{aligned} W_c &= 1000\, h \cdot F, \\ M_{CH} &= h \cdot a_i \cdot \beta \cdot K_1, \end{aligned} \qquad (14)$$

where h is the layer of flood run-off at P% coverage, mm, α_i is parameter of channeling, β is a coefficient which considers the influence of the agrotechnical background for the preceding year, k_1 is the coefficient of slope steepness. Parameters α_i and β are calculated using tables submitted in the literature (Anon. 1979). Inserting equation 14 in equation 13, we shall obtain.

$$C_{CH} = 10^6 a_1 \cdot B \cdot K_1 . \tag{15}$$

The data on liquid and solid run-off permit determination of removal of suspended particles, ammonium nitrogen, and nitrate of nitrogen. It is assumed that nitrate is removed with liquid run-off and ammonium nitrogen, with solid run-off.

Removal of nitrogen in run-off from farmland was measured by a method developed at VNIIVO (All-Union Scientific Research Institute of Water Protection) (Anon. 1981). Nitrate concentration in surface run-off per area F was calculated using the formula.

$$C^{NO_3} = \frac{K^{NO_3} P^c}{W_c} , \tag{16}$$

where K^{no} is the factor for scaling N to NO_3, $K^{no_3} = 4.4287$,

$$P^c = \frac{10^{-3}}{3} \frac{m h}{h_m(\Pi + 0,2 \frac{h}{H})} F \tag{17}$$

where P^c is removal of nitrates in liquid run-off, kg, m is amount of nitrates in plowed layer before rain, kg/ha, h_m is depth of plowed layer, m, Π is porosity of soil and H is layer of precipitations with P% coverage, mm.

Ammonium nitrogen concentration in surface run-off from the water catchment area is calculated using the following formula:

$$C^{NH_4} = \frac{K^{NH_4} P_T}{W_c} , \tag{18}$$

where K^{nh_4} is a factor for scaling N to NH_4 and is equal to 1.2857. P_T is removal of nitrogen with solid run-off, kg.

$$P_T = \frac{n_a M_{CH}}{\gamma h_m} F \cdot 10^{-4} , \tag{19}$$

where n_a is the quantity of metabolic-absorbed forms of nitrogen in the plowed layer by the time rains begin, kg/ha, M_{ch} is the modulus of sediment run-off (calculated using formula 14) and γ is volumetric weight of soil in tilled layer, tons/m^3. Thus, the model of formation of quality of surface run-off from farmed land consists of a set of submodels. the run-off submodels permits determination of the hydrograph of torrential water run-off, while submodels of water catchment area) of suspended sediments, ammonium nitrogen and nitrates.

The appropriate programs for a computer were developed for use of the above described submodels.

Let us discuss briefly determination of nominal conditions. According to the "Rules for protection of surface waters against sewage pollution," which are in effect in the USSR, calculation of volumes of diversion of sewage into water systems should be made for adverse hydrological conditions, to which average monthly water discharge with 95% coverage is usually related. However, the question of layer of rain run-off corresponding to this water flow rate in the river remained unanswered.

Analysis of formation of water quality in surface run-off from farm land revealed that the most hazardous concentrations of pollutants in the annual cycle are observed during the period of maximum rain activity. For this reason, in developing the nominal hydrological conditions for removal of pollutants from farmland, it was necessary to determine the probability of occurrence of two random processes together--maximum layer of rainflood run-off and mean summer-fall low-water level of discharge in rivers situated in zones with different moisture factors.

Studies of concurrent appearance of maximum layer of rain flood and minimum discharge of water with 95% coverage in the spring and fall low-water period revealed that a layer of rain run-off with 25% coverage (Kovalenko and Karabash 1983) should be taken as the nominal value.

As an example, let us estimate removal of ammonium nitrogen, nitrates and suspended particles in surface run-off from a hypothetical water catchment area (Figure 1). This water catchment has the following dimensions: 10 km on the y-axis and 20 km on the x-axis. The length of the river within the limits of the part of the catchment area in question is 27 km, and the entire river is 50 km long. Estimates of surface run-off were made using the following base data. H--layer of precipitations was considered to be 30 mm, n--coefficient of roughness for catchment slopes 0.2 and for thalwegs 0.02, r--rate of absorption was considered to be 0.20 mm/min and constant in time, Π--porosity of soil, 0.50, m--nitrate content in tilled layer 80 kg/ha, h_m--depth of tilled layer 0.2 m, n_a--ammonium nitrogen content in tilled layer 5 kg/ha, F--size of water catchment area 200 km^2.

On the basis of the developed programs, a geometrical model of the catchment area and run-off forming chains of triangles on the left and right banks of the stream, as well as run-off of torrential rain on the basis of the geometric model of a water catchment area, were constructed. To simplify input of information in calculating the concentraton of substances in the stream, several separate catchments, the run-off hydrographs of which are illustrated in Figure 2, were singled out in the model of the water catchment area. Run-off from the separate catchments 2 and 6 is given as being concentrated (over girder system) and from the others, deconcentrated (along stream) (Figure 3).

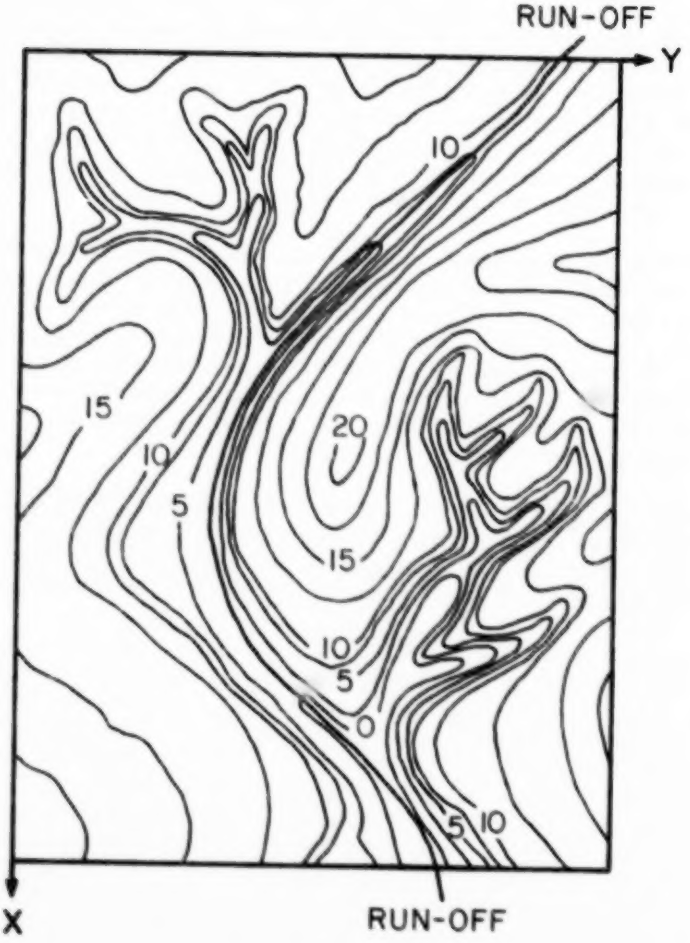

RUN-OFF
Y
10
15
10
5
20
15
10
5
0
5
10
X
RUN-OFF

Figure 1. Fragment of hypothetical water catchment area (horizontals, m).

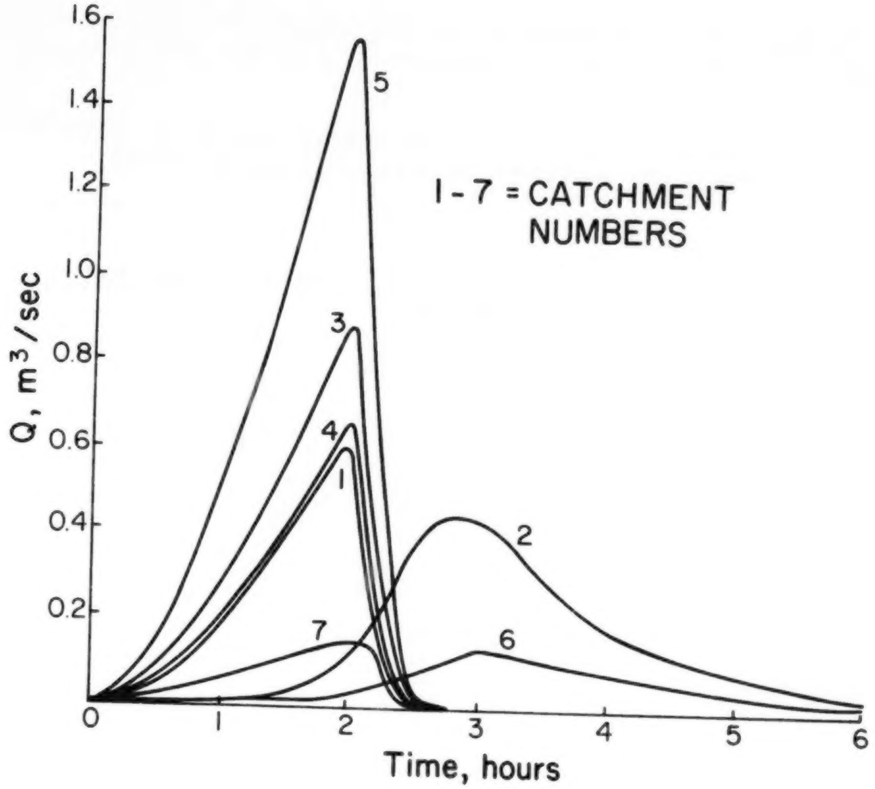


Figure 2. Calculated hydrographs of rain water runoff from separate catchments.

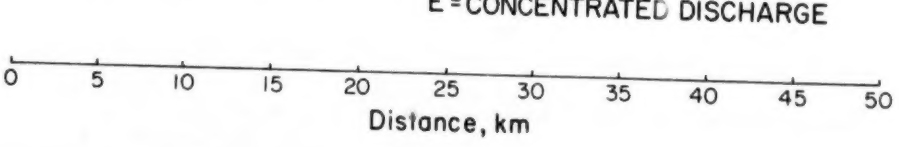

4
5
6
7
E
1
2
3
1-7 = CATCHMENT NUMBERS
E = CONCENTRATED DISCHARGE
0
5
10
15
20
25
30
35
40
45
50
Distance, km

Figure 3. Line diagram of river section.

Averaged water discharge of surface run-off and concentrations of suspended substances, ammonium nitrogen and nitrates are listed in Table 1.

TABLE 1. DISCHARGE OF RAIN RUN-OFF AND CONCENTRATIONS OF AMMONIUM NITROGEN, NITRATES AND SUSPENDED SUBSTANCES ENTERING STREAM.

Separate catchment number	Flow rate, m^3/s	Concentrations, mg/		
		Ammonium nitrogen	Nitrates	Suspended substances
1	0.26	0.15	74	1200
2	0.40	0.17	81	1400
3	0.35	0.13	67	1800
4	0.25	0.16	79	1350
5	0.64	0.13	65	1560
6	0.37	0.13	65	1350
7	0.06	0.14	70	1000

Formulas 3 were used to calculate concentrations of substances in river within the limits of the section studied, we used the formulas similar to the one for N_1 (3) to estimate the concentrations of suspended substances (N_5) under the conditions listed in Table 1, as well as with the following base data.

Background characteristics:

$Q = 40\ m^3/s$, $N_1 = 2\ mg/\ell$, $N_2 = 0.25\ mg/\ell$, $N_3 = 0.01\ mg/\ell$, $N_4 = 5\ mg/$,

$S = 10\ mg/\ell$ and $N_5 = 13\ mg/\ell$.

Characteristics of concentrated release E:

$Q = 0.3\ m^3/s$, $N_1 = 3.2\ mg/\ell$, $N_2 = 0.5\ mg/\ell$, $N_3 = 0.25\ mg/\ell$, $N_4 = 13\ mg/\ell$,

$S = 0\ mg/\ell$ and $N_5 = 100\ mm/\ell$.

Nonconservation coefficients.

$K_1 = 1 \cdot 10^{-6} 1/s$, $K_2 = 1.28 \cdot 10^{-7} 1/s$, $K_3 = 1.25 \cdot 10^{-4} 1/s$, $K_4 = 1.15 \cdot 10^{-7} 1/s$,

$K_s = 1.15 \cdot 10^{-5} 1/s$ and $K_5\ 2 \cdot 10^{-7} 1/s$.

Calculations were made in two variants. The first corresponds to lateral inflow of water as indicated in Table 1, and the second tripled lateral inflow, which also failed to lead to substantial change in concentration of substances in the flow of water. The results of calculations made in the first variant are illustrated in Figure 4, whereas Table 2 lists comparative concentrations of substances in typical sites of the river calculated by the two variants.

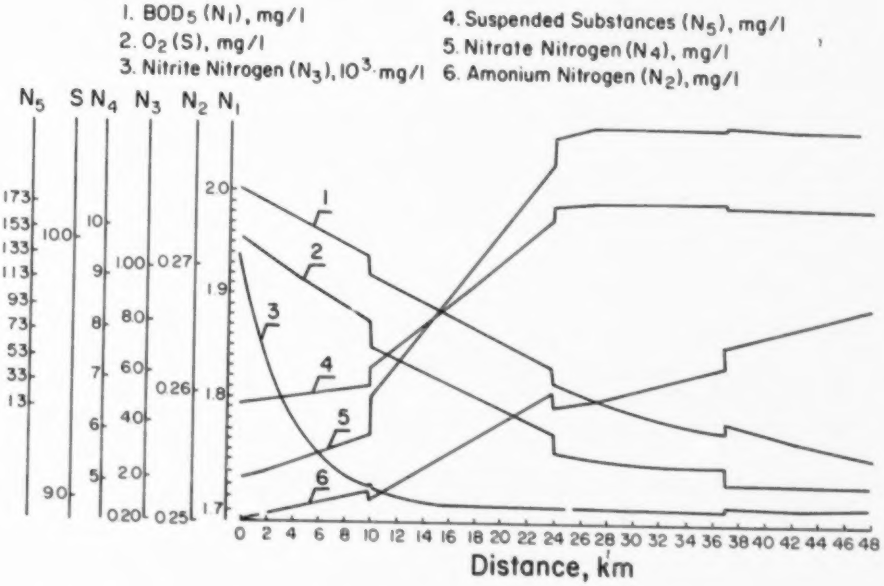


Figure 4. Calculated concentrations of substances in river current.

It must be noted that this approach permits not only consideration of the effect on water quality in the river of surface run-off, but allows an approach to optimization of water protection measures in a catchment provided the required amount of water is furnished in the river due to presence of elements of transitional matrix, if the link between intensity of water protection measures and cost indicators is known.

TABLE 2. CONCENTRATIONS OF SUBSTANCES IN TYPICAL SITES OF RIVER CURRENT WITH DIFFERENT LATERAL INFLOW

Calcul. variant No.	Distance from 1st sect., km	Concentration, mg/ℓ					
		N_1	$N_2 \cdot 10$	$N_3 \cdot 10^3$	N_4	$N_5 \cdot 10^{-1}$	S
1	10	1.94	2.52	1.27	5.81	2.67	9.58
	17	1.87	2.56	0.42	8.97	10.03	9.4

TABLE 2. Continued

	24	1.82	2.60	0.29	11.26	15.70	9.26
	27	1.60	2.59	0.26	11.88	17.07	9.14
	37	1.77	2.62	0.27	11.86	17.01	9.12
	50	1.73	2.67	0.28	11.84	16.90	9.05
2	10	1.89	2.50	1.02	7.63	5.93	9.46
	17	1.76	2.56	0.40	16.19	25.89	8.91
	24	1.68	2.63	0.29	22.30	41.06	8.67
	27	1.62	2.60	0.27	23.64	43.98	8.44
	37	1.59	2.62	0.26	23.60	43.82	8.54
	50	1.57	2.67	0.28	23.47	43.42	8.61

Key. N_1) BODs [biological oxygen demand]
N_2) ammonium nitrogen
N_3) nitrite nitrogen
N_4) nitrate nitrogen
N_5) suspended substances
S) dissolved oxygen

Note: Typical sites in the river were selected at the ends of the catchment sections before concentrated releases and at the end of the nominal section.

BIBLIOGRAPHY

Anon. 1975. Metodicheskiye rekomendatsii po uchetu poverkhnostnogo stoka i smyva pochv pri izuchenii vodnoy erozii (Methodological Recommendations on How to Keep a Record of Surface Run-Off and Soil Wash-Off in Studies of Aquatic Erosion). Leningrad, USSR. 88.

Anon. 1979. Instruktsiya po opredeleniye raschetnykh gidrologicheskikh kharakteristik pri proyektirovanii protivoerozionnykh meropriyatiy na Yevropeyskoy territorii SSSR (Instructions for Determining Nominal Hydrological Characteristics in Planning Anti-Erosion Measures in European USSR). Leningrad, USSR. 63 p.

Anon. 1981. Metodika otsenki vynosa pestitsidov i biogennykh veshchestv neorganizovannym poverkhnostnym stokom sel'skokhozyaystvennykh ugodiy bogarnogo zemledeliya (Methods of Evaluating Removal of Pesticides and Biogenous Substances in Unorganized Surface Run-Off From Land Farmed by the Nonirrigated Method). Kharkov, USSR. 64 p.

Aytsam, A. M., Kh. A. Vel'ner, and L. L. Paal'. 1968. Calculation of changes in pollutant concentrations in rivers, Gigiyena i Sanitariya. 11:3-10.

Kovalenko, M S. and G. A. Karabash. 1983. Evaluation of effect of removal of pesticides in surface run-off on water quality in streams. In. Okhrana vody ot zagryazneniya poverkhnostnym stokom (Protection of Water Against Pollution by Surface Run-Off). Kharkov, USSR. pp 80-92.

Koval'cyuk, P. I., B. A. Akisin, V. L. Pavelko, and A. A. Matveyev. 1979. Synthesis of models of transformation of nonconservation substances in streams during diversions in a system of field observations. Gidrokh. Mater. 72:111-115.

Kuchment, L. S., V. N. Demidov, and Yu. G. Motovilov 1983. Formirovaniye rechnogo stoka (Formation of River Run-Off). Moscow, USSR. 216.

Nash, J. E. 1957. The form of the instantaneous unit hydrograph. 3(45):14-121.

Yeremenko, Ye. V., Yu. M. Plis, and N. I. Selyuk. 1981. Model of water quality in Connecticut and North Donets Rivers. In: Metodologiya i praktika planirovaniya okhrany vod rechnykh basseynov (Methodology and Practice of Planning Protection for River Basin Waters). Kharkov, USSR. pp 126-170.

APPENDIX

Values for elements of transition matrices in solutions of (3)

$$G_{11} = \frac{Q_0}{Q} exp(-\kappa_1 x/V);$$

$$G_1 = \frac{1}{Q}\left[N_1^p(Q - Q_{cp}) - N_1^p(Q_0 - Q_{cp}) exp(-\kappa_1 x/V)\right];$$

$$G_{22} = \frac{Q_0}{Q} exp(-\kappa_2 x/V);$$

$$G_{21} = \frac{Q_0}{Q} \cdot \frac{\delta \kappa_1}{\kappa_2 - \kappa_1}\left(exp(-\kappa_1 x/V) - exp(-\kappa_2 x/V)\right);$$

$$G_2 = \frac{1}{Q}\left\{N_2^p(Q - Q_{cp}) - \frac{\delta \kappa_1}{\kappa_2 - \kappa_1} N_1^p(Q_0 - Q_{cp}) exp(-\kappa_1 x/V) + \right.$$

$$\left. + \left[-N_2^p(Q_0 - Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2 - \kappa_1} N_1^p(Q_0 - Q_{cp})\right] exp(-\kappa_2 x/V)\right\};$$

$$G_{33} = \frac{Q_0}{Q} \exp(-\kappa_3 x/V);$$

$$G_{32} = \frac{Q_0}{Q} \frac{\kappa_2}{\kappa_3 - \kappa_2} \left(\exp(-\kappa_2 x/V) - \exp(-\kappa_3 x/V) \right);$$

$$G_{31} = \frac{Q_0}{Q} \frac{\delta \kappa_1}{\kappa_2 - \kappa_1} \left[\frac{\kappa_2}{\kappa_3 - \kappa_1} \exp(-\kappa_1 x/V) - \frac{\kappa_2}{\kappa_3 - \kappa_2} \exp(-\kappa_2 x/V) - \right.$$

$$\left. - \left(\frac{\kappa_2}{\kappa_3 - \kappa_1} - \frac{\kappa_2}{\kappa_3 - \kappa_2} \right) \exp(-\kappa_3 x/V) \right];$$

$$G_3 = \frac{1}{Q} \left\{ N_3^p (Q - Q_{\text{ср}}) - \frac{\delta \kappa_1 \kappa_2}{(\kappa_2 - \kappa_1)(\kappa_3 - \kappa_1)} N_1^p (Q_0 - Q_{\text{ср}}) \exp(-\kappa_1 x/V) + \right.$$

$$+ \frac{\kappa_2 \cdot}{\kappa_3 - \kappa_2} \left[-N_2^p (Q_0 - Q_{\text{ср}}) + \frac{\delta \kappa_1}{\kappa_2 - \kappa_1} N_1^p (Q_0 - Q_{\text{ср}}) \right] \exp(-\kappa_2 x/V) +$$

$$+ \left[-N_3^p (Q_0 - Q_{\text{ср}}) + \frac{\delta \kappa_1 \kappa_2}{(\kappa_2 - \kappa_1)(\kappa_3 - \kappa_1)} N_1^p (Q_0 - Q_{\text{ср}}) - \right.$$

$$\left. \left. - \frac{\kappa_2}{\kappa_3 - \kappa_2} \left[-N_2^p (Q_0 - Q_{\text{ср}}) + \frac{\delta \kappa_1}{\kappa_2 - \kappa_1} N_1^p (Q_0 - Q_{\text{ср}}) \right] \right] \exp(-\kappa_3 x/V) \right\};$$

$$G_{44} = \frac{Q_0}{Q} \exp(-\kappa_4 x/V);$$

$$G_{43}=\frac{Q_0}{Q}\,\frac{\kappa_3}{\kappa_4-\kappa_3}\left(exp(-\kappa_3 x/v)-exp(-\kappa_4 x/v)\right);$$

$$G_{42}=\frac{Q_0}{Q}\,\frac{\kappa_2}{\kappa_3-\kappa_2}\left[\frac{\kappa_3}{\kappa_4-\kappa_2}\,exp(-\kappa_2 x/v)-\frac{\kappa_3}{\kappa_4-\kappa_3}\,exp(-\kappa_3 x/v)-\right.$$

$$\left.-\left(\frac{\kappa_3}{\kappa_4-\kappa_2}-\frac{\kappa_3}{\kappa_4-\kappa_3}\right)exp(-\kappa_4 x/v)\right];$$

$$G_{41}=\frac{Q_0}{Q}\,\frac{\delta\kappa_1}{\kappa_2-\kappa_1}\left\{\frac{\kappa_2\ \kappa_3}{(\kappa_3-\kappa_1)(\kappa_4-\kappa_1)}\,exp(-\kappa_1 x/v)-\frac{\kappa_2\ \kappa_3}{(\kappa_3-\kappa_2)(\kappa_4-\kappa_2)}\times\right.$$

$$\times\, exp(-\kappa_2 x/v)-\frac{\kappa_3}{\kappa_4-\kappa_3}\left(\frac{\kappa_2}{\kappa_3-\kappa_1}-\frac{\kappa_2}{(\kappa_3-\kappa_2)}\right)exp(-\kappa_3 x/v)-$$

$$-\left[\frac{\kappa_2\ \kappa_3}{(\kappa_3-\kappa_1)(\kappa_4-\kappa_1)}-\frac{\kappa_2\ \kappa_3}{(\kappa_3-\kappa_2)(\kappa_4-\kappa_2)}-\frac{\kappa_3}{\kappa_4-\kappa_3}\left(\frac{\kappa_2}{\kappa_3-\kappa_1}-\right.\right.$$

$$\left.\left.\left.-\frac{\kappa_2}{\kappa_3-\kappa_2}\right)\right]exp(-\kappa_4 x/v)\right\};$$

$$G_4=\frac{1}{Q}\left\{N_4^p(Q-Q_{cp})-\frac{\delta\kappa_1\ \kappa_2\ \kappa_3}{(\kappa_2-\kappa_1)(\kappa_3-\kappa_1)(\kappa_4-\kappa_1)}\,N_1^p(Q_0-Q_{cp})\,exp(-\kappa_1 x/v)+\right.$$

$$+\frac{\kappa_2\ \kappa_3}{(\kappa_3-\kappa_2)(\kappa_4-\kappa_2)}\left[-N_2^p(Q_0-Q_{cp})+\frac{\delta\kappa_1}{\kappa_2-\kappa_1}N_1^p(Q_0-Q_{cp})\right]exp(-\kappa_2 x/v)+$$

$$+\frac{k_3}{k_4-k_3}\Big[-N_3^p(Q_0-Q_{cp})+\frac{\delta k_1 k_2}{(k_2-k_1)(k_3-k_1)}N_1^p(Q_0-Q_{cp})-\frac{k_2}{k_3-k_2}\times$$

$$\times\Big[-N_2^p(Q_0-Q_{cp})+\frac{\delta k_1}{k_2-k_1}N_1^p(Q_0-Q_{cp})\Big]\Big]\exp(-k_3 x/V)+\Big\{-N_4^p(Q_0-Q_{cp})+$$

$$+\frac{\delta k_1 k_2 k_3}{(k_2-k_1)(k_3-k_1)(k_4-k_1)}N_1^p(Q_0-Q_{cp})-\frac{k_2 k_3}{(k_3-k_2)(k_4-k_2)}\Big[-N_2^p(Q_0-Q_{cp})+$$

$$+\frac{\delta k_1}{k_2-k_1}N_1^p(Q_0-Q_{cp})\Big]-\frac{k_3}{k_4-k_3}\Big[-N_3^p(Q_0-Q_{cp})+\frac{\delta k_1 k_2}{(k_2-k_1)(k_3-k_1)}N_1^p\times$$

$$\times(Q_0-Q_{cp})-\frac{k_2}{k_3-k_2}\Big[-N_2^p(Q_0-Q_{cp})+\frac{\delta k_1}{k_2-k_1}N_1^p(Q_0-Q_{cp})\Big]\Big]\Big\}\exp(-k_4 x/V)\Big\};$$

$$G_{ss}=\frac{Q_0}{Q}\exp(-k_s x/V);$$

$$G_{s3}=\frac{Q_0}{Q}\frac{k_3}{k_s-k_3}\gamma_3\big(\exp(-k_s x/V)-\exp(-k_3 x/V)\big);$$

$$G_{s2}=\frac{Q_0}{Q}\Big\{\gamma_2\frac{k_2}{k_s-k_2}\big(\exp(-k_s x/V)-\exp(-k_2 x/V)\big)+\gamma_3\frac{k_2}{k_3-k_2}\times$$

$$\times\Big[\frac{k_3}{k_s-k_2}\big(\exp(-k_s x/V)-\exp(-k_2 x/V)\big)-\frac{k_3}{k_s-k_3}\big(\exp(-k_s x/V)-$$

$$- exp(-k_3 x/v)\Big)\Big]\Big]\Big\};$$

$$G_{g1} = \frac{Q_0}{Q}\Big\{\gamma_1 \frac{k_1}{k_g - k_1}\big(exp(-k_g x/v) - exp(-k_1 x/v)\big) + \gamma_2 \frac{\delta k_1}{k_2 - k_1} \times$$

$$\times \Big[\frac{k_2}{k_g - k_1}\big(exp(-k_g x/v) - exp(-k_1 x/v)\big) - \frac{k_2}{k_g - k_2}\big(exp(-k_g x/v) -$$

$$- exp(-k_2 x/v)\big)\Big] + \gamma_3 \frac{\delta k_1}{k_2 - k_1}\Big[\frac{k_2 k_3}{(k_3 - k_1)(k_g - k_1)}\big(exp(-k_g x/v) -$$

$$- exp(-k_1 x/v)\big) - \frac{k_2 k_3}{(k_3 - k_2)(k_g - k_2)}\big(exp(-k_g x/v) - exp(-k_2 x/v)\big) +$$

$$+ \frac{k_3}{k_g - k_3}\Big(\frac{k_2}{k_3 - k_2} - \frac{k_2}{k_3 - k_1}\Big)\big(exp(-k_g x/v) - exp(-k_3 x/v)\big)\Big]\Big\}$$

$$G_g = \frac{1}{Q}\Big\{S^p(Q - Q_{\text{ср}}) + S_{\text{н}} Q_{\text{ср}} + \gamma_1 \frac{k_1}{k_g - k_1} N_1^p (Q_0 - Q_{\text{ср}}) exp(-k_1 x/v) -$$

$$- \gamma_2\Big[-\frac{\delta k_1 k_2}{(k_2 - k_1)(k_g - k_1)} N_1^p (Q_0 - Q_{\text{ср}}) exp(-k_1 x/v) + \frac{k_2}{k_g - k_2}\Big[-N_2^p (Q_0 - Q_{\text{ср}}) +$$

$$+ \frac{\delta k_1}{k_2 - k_1} N_1^p (Q_0 - Q_{\text{ср}})\Big] exp(-k_2 x/v)\Big] - \gamma_3\Big\{-\frac{\delta k_1 k_2 k_3}{(k_2 - k_1)(k_3 - k_1)(k_g - k_1)} N_1^p (Q_0 - Q_{\text{ср}}) \times$$

$$\times \exp(-\kappa_1 x/V) + \frac{\kappa_2 \ \kappa_3}{(\kappa_3-\kappa_2)(\kappa_s-\kappa_2)}\left[-N_2^p(Q_0-Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2-\kappa_1}N_1^p(Q_0-Q_{cp})\right]\times$$

$$\times \exp(-\kappa_2 x/V) + \frac{\kappa_3}{\kappa_s-\kappa_3}\left[-N_3^p(Q_0-Q_{cp}) + \frac{\delta \kappa_1 \ \kappa_2}{(\kappa_2-\kappa_1)(\kappa_3-\kappa_1)}N_1^p(Q_0-Q_{cp}) - \frac{\kappa_2}{\kappa_3-\kappa_2}\times\right.$$

$$\left.\times\left[-N_2^p(Q_0-Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2-\kappa_1}N_1^p(Q_0-Q_{cp})\right]\right]\exp(-\kappa_3 x/V)\Bigg\} + \Bigg\{-S^p(Q_0-Q_{cp}) - S_{н}Q_{cp} -$$

$$-\gamma_1 \frac{\kappa_1}{\kappa_s-\kappa_1}N_1^p(Q_0-Q_{cp}) + \gamma_2\left[-\frac{\delta \kappa_1 \ \kappa_2}{(\kappa_2-\kappa_1)(\kappa_s-\kappa_1)}N_1^p(Q_0-Q_{cp}) + \frac{\kappa_2}{\kappa_s-\kappa_2}\left[-N_2^p\times\right.\right.$$

$$\left.\left.\times(Q_0-Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2-\kappa_1}N_1^p(Q_0-Q_{cp})\right]\right] + \gamma_3\Bigg\{-\frac{\delta \kappa_1 \ \kappa_2 \ \kappa_3}{(\kappa_2-\kappa_1)(\kappa_3-\kappa_1)(\kappa_s-\kappa_1)}N_1^p(Q_0-Q_{cp}) +$$

$$+\frac{\kappa_2 \ \kappa_3}{(\kappa_3-\kappa_2)(\kappa_s-\kappa_2)}\left[-N_2^p(Q_0-Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2-\kappa_1}N_1^p(Q_0-Q_{cp})\right] + \frac{\kappa_3}{\kappa_s-\kappa_3}\left[-N_3^p(Q_0-Q_{cp}) +\right.$$

$$+\frac{\delta \kappa_1 \ \kappa_2}{(\kappa_2-\kappa_1)(\kappa_3-\kappa_1)}N_1^p(Q_0-Q_{cp}) - \frac{\kappa_2}{\kappa_3-\kappa_2}\left[-N_2^p(Q_0-Q_{cp}) + \frac{\delta \kappa_1}{\kappa_2-\kappa_1}N_1^p\times\right.$$

$$\left.\left.\times(Q_0-Q_{cp})\right]\right]\Bigg\}\Bigg\}\exp(-\kappa_s x/v)\Bigg\};$$

where $$Q_{cp} = \frac{1}{x}\int^{x} Q\,dx\,; \quad Q = Q_0 + Q^p(x).$$

Translator's note: Subscript "cp" is Russian abbreviationfor average or mean.

CONTROL OF URBAN NONPOINT SOURCES IN GREAT LAKES BASIN

by

D. Athayde, P. Bubar, and J. Meek[1]

ABSTRACT

The Nationwide Urban Runoff Program (NURP) was conducted by the U.S. Environmental Protection Agency (EPA) and many cooperating federal, state, regional, and local agencies which were distributed across the United States. The program consisted of individual project studies that were overseen by a technical team at EPA. The program was managed centrally because it covered a broad spectrum of technical planning issues at many geographic locations, several of which were in the Great Lakes Basin. In 1982, the International Joint Commission (IJC) established a Nonpoint Source Control Task Force to review and evaluate the effectiveness of pollution reduction activities recommended by the commission. An August 1983 report provided an overview of the extent of implementation and the effectiveness of various nonpoint source programs in the Great Lakes Basin. This paper summarizes the results of the NURP projects in the Great Lakes Basin and compares them to the findings of the IJC's Nonpoint Source Task Force.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

The Nationwide Urban Runoff Program (NURP) was initiated as part of the water quality planning process required under Section 208 of the U.S. Clean Water Act of 1977. The program began in 1978 and concluded in 1983. NURP had six projects in four states within the Great Lakes Basin out of a total of 28 demonstration projects participating. Results from five of those six projects are now available and will be discussed in this paper.

---

[1] Office of Water, U.S. Environmental Protection Agency, Washington DC 20460 USA

The NURP was conducted by the U.S. Environmental Protection Agency (EPA) and many cooperating federal, state, regional, and local agencies distributed widely across the United States. The program consisted of individual project studies that were overseen by a technical team at EPA. The program was managed centrally because it covered a broad spectrum of technical and planning issues at many geographic locations, several of which were in the Great Lakes Basin.

The program was developed, implemented and managed by the Office of Water's Water Planning Division at EPA with additional contributions from EPA's Office of Research and Development and the EPA regional offices. The data summary and analysis was done mainly by a team of consultants under contract with EPA.

In 1972, the Pollution from Land Use Reference Group (PLUARG) of the International Joint Commission (IJC) was established for the purpose of determining the levels and causes of pollution from land use activities in the Great Lakes Basin. PLUARG reported its findings and recommendations to the IJC in 1978. Subsequently, the IJC forwarded a set of recommendations to the U.S. and Canadian governments in 1980.

In January 1982, the Water Quality Programs Committee of the IJC's Water Quality Board recommended that a Nonpoint Source Control Task Force be established. Consequently, the Water Quality Board set up a 14-member Task Force (seven members each from the United States and Canada). The Task Force was to review and evaluate the effectiveness of the activities recommended by the IJC to reduce nonpoint source pollution. The Task Force did this and prepared a report in August 1983. The report provided an overview of the extent of implementation and the effectiveness of various nonpoint source programs in the Great Lakes Basin.

This paper summarizes the results of the NURP projects in the Great Lakes Basin and compares them to the findings of the IJC's Nonpoint Source Task Force.

BACKGROUND OF NATIONWIDE URBAN RUNOFF PROGRAM

The NURP was begun in 1978 in an effort to provide the U.S. government with answers to questions concerning urban runoff including: (1) Is it a problem? (2) What are its effects on receiving waters? and (3) What control mechanisms are available?

The program consisted of 28 projects for collecting and analyzing data in an effort to answer the local questions that existed as well as to provide information to U.S. Environmental Protection Agency (EPA). Because there were local objectives that had to be met, EPA played a strong management role to ensure that each project was not off on its own tangent. This role called for providing technical assistance in work plan development, in data collection and instrumentation programs, in data analysis, and in report writing. The result of this approach was 28 projects working separately but also within the direction of the NURP team.

A major objective of the NURP was the acquisition of data. The data would be used to characterize the urban runoff problem, evaluate receiving water impacts, and evaluate management practices. Because of this, quality assurance and quality control were important elements in each project. There was considerable effort to ensure that the NURP data were valid. Questionable data would be of no use.

NURP collected data on an event basis with anywhere from 12 to 30 events at one site. Both discrete samples and composite samples were collected, usually by an automatic sampler. Rainfall volume, runoff volume, and runoff quality data were collected from a number of urban catchments in each city. The event mean concentration (EMC), defined as the total constituent mass discharge divided by the total runoff volume, was chosen as the primary measure of the pollutant load. EMCs were calculated for each event at each site in the accessible data base. If a flow-weighted composite sample was taken, its concentration was used to represent the EMC. Where sequential discrete samples were taken over the hydrograph, the EMC was determined by calculating the area under the loadograph (the curve of concentration times discharge rate over time) and dividing it by the area under the hydrograph (the curve of runoff volume over time). For the purpose of determining EMCs, rainfall events were defined to be separate precipitation events when there was an intervening time period of at least 6 hours without rain.

EMCs were chosen as the primary water quality characteristic subjected to detailed analysis, even though it is recognized that mass loading characteristics of urban runoff (e.g., pounds/acre for a specified time interval) is ultimately the relevant factor in many situations. The reason is that, unlike EMCs, mass loadings are strongly influenced by the amount of precipitation and runoff, and estimates of typical annual mass loads will be biased by the size of monitored storm events. The most reliable basis for characterizing annual or seasonal mass loads is using EMCs and site-specific rainfall/runoff characteristics. In addition, EMCs were chosen because we were not interested in looking at one storm event but rather many storm events over long periods of time. What occurs during one storm event held little interest to us.

A statistical approach was adopted for characterizing the properties of EMCs for standard pollutants. Standard statistical procedures were used to define the probability distribution, central tendency (a mean or median) and spread (standard deviation or coefficient of variation) of EMC data. EMC data for each pollutant from all storms and monitoring sites were compiled in a central data base management system at EPA.

The underlying probability distribution of the EMC data was examined and tested by the NURP team and consultants, by both visual and statistical methods. With relatively few isolated exceptions, it was found that the probability distribution of EMCs at individual sites can be characterized by log-normal distributions. Because of this, the log (base e) transforms of all urban runoff data were used in developing the statistical characterizations.

In addition to summarizing and analyzing the concentration data, several management control practices were evaluated and analyzed. The management practices analyzed were the use of detention basins and street sweeping. Detention basins were also analyzed by the Nonpoint Source Control Task Force.

SUMMARY OF NURP PROJECTS IN GREAT LAKES BASIN

ASSESSMENT OF CONCENTRATIONS AND LOADS IN NURP PROJECTS

As mentioned previously, six projects participating in the NURP were located in the Great Lakes Basin. Five of the six project's data have been analyzed by the NURP. These five areas were Ann Arbor, Michigan; Lansing, Michigan; Glen Ellyn, Illinois; Milwaukee, Wisconsin; and Rochester, New York (see Figure 1).

The data analysis focused on ten pollutants, which were selected after initial screening of the data. The pollutants were total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand(COD), total phosphorus (as P)(TP), soluble phosphorus (as P) (SP), total kjeldahl nitrogen (as N) (TKN), N - nitrite + nitrate (as N) (NO_{2+3}), total copper (Cu), total lead (Pb), and total zinc (Zn).

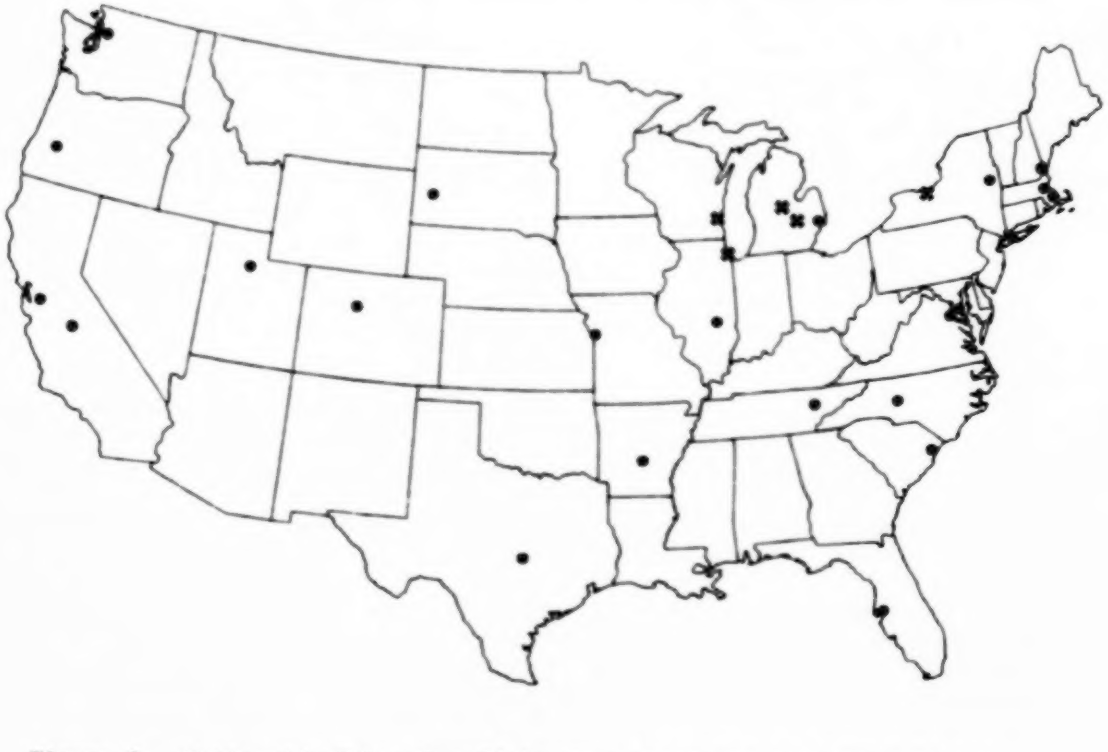


Figure 1. Nationwide Urban Runoff Program locations in United States

The NURP analysis summarized the statistical parameters--mean, median, and coefficient of variation. The data from the 22 sites in the 5 representative NURP projects are shown in Table 1.

TABLE 1. STATISTICAL PARAMETERS FOR 22 SITES IN GREAT LAKES NURP

Pollutant and Project*	No. observ.	Mean	Coeffic. variation	Median	90 percent confidence limits
Total Susp. Solids (μg/1)					
NY3	10	134	1.15	88	52-150
NY3	7	294	1.12	196	85-263
WI1	45	266	0.44	243	219-270
WI1	33	170	0.68	141	117-169
WI1	23	251	0.69	206	165-258
IL2	27	250	0.75	200	161-249
NY3	11	154	0.92	113	74-173
NY3	9	63	0.74	51	34-77
MI3	5	33	0.77	26	14-50
MI1	35	85	1.28	52	39-69
MI1	23	158	1.26	98	69-139
MI3	6	46	0.37	43	32-58
MI3	6	68	0.47	61	42-88
MI1	23	172	0.85	131	101-171
MI3	5	80	0.91	59	28-124
WI1	47	383	0.78	302	255-357
WI1	58	212	0.86	161	131-197
WI1	42	202	0.68	167	142-196
NY3	12	141	0.76	112	79-159
WI1	29	412	0.97	296	229-382
MI1	18	92	0.82	71	53-95
MI1	20	188	0.94	137	101-186
Total Phosphorus (μg/1)					
NY3	13	301	0.54	265	206-340
NY3	8	448	0.47	405	300-456
WI1	45	229	0.45	209	188-233
WI1	35	258	0.51	230	201-264
WI1	23	453	0.69	373	298-466
IL2	26	506	0.79	397	314-501
NY3	13	193	0.46	175	141-217
NY3	12	195	0.47	177	140-223
MI3	5	91	0.38	85	60-121
MI1	35	198	0.64	167	141-197
MI1	22	458	0.65	384	309-477
MI3	6	103	0.50	93	63-137
MI3	6	268	0.47	243	168-351
MI1	23	394	0.54	347	285-410

TABLE 1 (cont'd). STATISTICAL PARAMETETERS FOR 22 SITES IN GREAT LAKES NURP

Pollutant and Project*	No. observ.	Mean	Coeffic. variation	Median	90 percent confidence limits
MI3	5	134	0.56	117	71-193
WI1	47	289	0.59	249	218-284
WI1	60	108	0.56	94	84-105
WI1	44	105	0.79	82	69-98
NY3	12	216	0.26	209	183-239
WI1	29	511	1.19	330	245-443
MI1	18	546	0.58	472	378-589
MI1	17	435	0.71	355	271-465
Soluble Phosphorus (µg/l)					
NY3	0	-	-	-	-
NY3	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
IL2	24	98	1.21	63	45-88
NY3	0	-	-	-	-
NY3	0	-	-	-	-
MI3	5	33	0.55	29	18-47
MI1	32	43	0.76	34	28-42
MI1	20	68	0.68	56	44-71
MI3	6	13	0.37	13	10-17
MI3	6	59	0.88	44	24-82
MI1	21	47	0.47	42	45-50
MI3	5	39	0.46	35	23-53
WI1	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
NY3	0	-	-	-	-
WI1	0	-	-	-	-
MI1	14	127	0.72	103	76-140
MI1	16	59	1.24	37	24-56
TKN (µg/l)					
NY3	13	1492	0.45	1358	1098-1679
NY3	7	3246	0.90	2411	1369-4245
WI1	45	1260	0.50	1125	908-1395
WI1	15	1102	0.54	969	801-1173
WI1	1	-	-	-	-
IL2	0	-	-	-	-
NY3	13	1099	0.50	982	778-1240
NY3	10	1111	0.36	1045	854-1279
MI3	5	889	0.11	883	796-981
MI1	35	1490	0.53	1316	1142-1516

TABLE 1 (cont'd). STATISTICAL PARAMETERS FOR 22 SITES IN GREAT LAKES NURP

Pollutant and Project*	No. observ.	Mean	Coeffic. variation	Median	90 percent confidence limits
MI1	23	1631	0.42	1506	1304-1740
MI3	6	845	0.29	811	642-1025
MI3	6	1056	0.22	1031	862-1233
MI1	23	1988	0.47	1802	1536-2115
MI3	5	1116	0.15	1104	958-1273
WI1	16	1452	0.35	1369	1180-1589
WI1	27	1023	0.44	936	815-1075
WI1	25	1073	0.61	916	755-1110
NY3	13	1256	0.45	1144	925-1414
WI1	8	1656	0.65	1389	933-2068
MI1	18	1274	0.57	1107	891-1376
MI1	18	1713	0.56	1493	1205-1850
Nitrite plus nitrate (μg/l)					
NY3	0	-	-	-	-
NY3	0	-	-	-	-
WI1	18	775	0.48	699	580-843
WI1	24	625	0.39	582	510-664
WI1	3	-	-	-	-
IL2	21	796	0.55	699	576-848
NY3	0	-	-	-	-
NY3	0	-	-	-	-
MI3	5	1108	0.17	1092	930-1283
MI1	35	775	0.49	696	192-558
MI1	23	883	0.44	807	694-938
MI3	6	284	0.48	256	176-372
MI3	5	469	0.24	456	364-571
MI1	23	875	0.43	803	693-931
MI3	5	1033	0.76	821	431-1563
WI1	17	751	0.69	618	474-805
WI1	28	708	0.68	584	479-712
WI1	26	781	0.69	642	520-791
NY3	0	-	-	-	-
WI1	12	783	0.50	702	549-897
MI1	18	686	0.40	637	544-746
MI1	17	742	0.52	657	534-808
Total copper (μg/l)					
NY3	0	-	-	-	-
NY3	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-

TABLE 1 (cont'd). STATISTICAL PARAMETERS FOR 22 SITES IN GREAT LAKES NURP

Pollutant and Project*	No. observ.	Mean	Coeffic. variation	Median	90 percent confidence limits
IL2	26	49	0.53	43	36-51
NY3	0	-	-	-	-
NY3	0	-	-	-	-
MI3	0	-	-	-	-
MI1	16	15	0.54	13	10-16
MI1	13	30	0.63	26	20-35
MI3	0	-	-	-	-
MI3	0	-	-	-	-
MI1	9	14	0.31	13	11-16
MI3	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
WI1	0	-	-	-	-
NY3	0	-	-	-	-
WI1	0	-	-	-	-
MI1	6	36	0.53	32	21-48
MI1	7	25	0.65	21	14-32
Total lead (μg/1)					
NY3	13	34	0.77	27	19-38
NY3	87	193	0.89	144	86-240
WI1	44	95	0.72	77	65-91
WI1	35	108	0.67	90	75-107
WI1	22	303	1.14	200	143-280
IL2	24	322	1.01	227	169-304
NY3	10	12	0.42	11	9-14
NY3	12	35	1.65	18	10-33
MI3	0	-	-	-	-
MI1	24	111	1.09	75	55-102
MI1	18	122	0.90	91	66-125
MI3	6	21	1.63	11	4-28
MI3	5	61	0.71	50	27-92
MI1	18	170	1.39	99	65-151
MI3	4	-	-	-	-
WI1	45	582	0.94	424	348-517
WI1	59	193	0.83	148	126-173
WI1	44	121	0.73	98	83-115
NY3	13	47	0.50	42	33-53
WI1	27	409	0.86	310	243-396
MI1	13	116	0.77	92	66-129
MI1	13	115	0.76	92	66-128

TABLE 1 (cont'd). STATISTICAL PARAMETERS FOR 22 SITES IN GREAT LAKES NURP

Pollutant and Project*	No. observ.	Mean	Coeffic. variation	Median	90 percent confidence limits
Total zinc (μg/1)					
NY3	9	415	0.88	312	195-499
NY3	8	488	1.10	327	180-594
WI1	18	106	1.34	63	42-95
WI1	21	108	1.20	69	49-99
WI1	0	-	-	-	-
IL2	27	230	0.69	189	154-232
NY3	9	792	2.39	306	130-720
NY3	9	1063	3.14	322	124-839
MI3	2	-	-	-	-
MI1	17	121	0.45	110	92-132
MI1	14	245	0.71	200	148-271
MI3	4	-	-	-	-
MI3	4	-	-	-	-
MI1	9	149	0.35	140	113-173
MI3	2	-	-	-	-
WI1	27	476	1.21	303	222-414
WI1	32	145	1.16	94	71-124
WI1	19	156	0.75	125	96-163
NY3	9	1416	2.55	517	214-1247
WI1	7	280	0.66	234	150-363
MI1	7	244	0.42	225	167-303
MI1	27	223	0.54	196	135-284

*Site description:

Proj.	Site	Land use %	Area (acres)	Pop. Dens. (no./acre)	% Imp.
NY3	Cranston	100% Res.	166	5	22
NY3	E. Roch.	100% Res.	346	18	38
WI1	Burbank	100% Res.	63	15	50
WI1	Hastings	100% Res.	33	17	51
WI1	Lincoln	97% Res.	36	18	57
IL2	Comb. Inl	85% Res.	524	8	17
NY3	Thornell	100% Open	28,416	-	4
NY3	Thomas Cr	91% Open	17,728	1	11
MI3	Traver	90% Open	2,303	-	6
MI1	Waverly	Mixed	30	11	68
MI1	Grand R.	Mixed	453	5	38
MI3	Pitt AAS	Mixed	2,001	2	21
MI3	Pitt AAN	Mixed	2,871	7	26
MI1	Grace N	Mixed	164	5	28
MI3	Swift Run	Mixed	1,207	2	4
WI1	Wood Ctr	Mixed	45	12	81
WI1	Post Off	100% Comm	12	0	100

TABLE 1 (cont'd). STATISTICAL PARAMETERS FOR 22 SITES IN GREAT LAKES NURP

*Site description (cont'd):

Proj.	Site	Land use %	Area (acres)	Pop. Dens. (no./acre)	% Imp.
WI1	Rustler	100% Comm	12	0	100
NY3	Southgate	100% Comm	179	2	21
WI1	State Fr	74% Comm	29	10	77
MI1	Indus Dr	100% Ind	63	0	64
MI1	Grace S.	52% Ind	75	5	39

The runoff coefficient statistics also were calculated for the represented sites (Table 2).

TABLE 2. RUNOFF COEFFICIENT STATISTICS FOR GREAT LAKES NURP SITES

							Runoff Coeffic.	
Proj.	Site	Land use %	Drainage area (acres)	Pop. dens. (no./acre)	% Imp.	No. obs.	Median	Coeff variation
NY3	Cranston	100% Res.	166	5	22	13	0.16	0.33
NY3	E. Roch.	100% Res.	346	18	38	9	0.20	0.42
WI1	Burbank	100% Res.	63	15	50	44	0.27	0.92
WI1	Hastings	100% Res.	33	17	51	33	0.27	0.37
WI1	Lincoln	97% Res.	36	18	57	19	0.38	0.55
IL2	Comb. Inl	85% Res.	524	8	17	29	0.17	0.48
NY3	Thornell	100% Open	28,416	–	4	13	0.06	0.93
NY3	Thomas Cr	91% Open	17,728	1	11	13	0.04	0.56
MI3	Traver	90% Open	2,303	–	6	5	0.11	1.07
MI1	Waverly	Mixed	30	11	68	35	0.36	0.25
MI1	Grand R.	Mixed	453	5	38	23	0.11	0.50
MI3	Pitt AAS	Mixed	2,001	2	21	6	0.19	0.46
MI3	Pitt AAN	Mixed	2,871	7	26	6	0.10	0.43
MI1	Grace N	Mixed	164	5	28	23	0.11	0.41
MI3	Swift Run	Mixed	1,207	2	4	5	0.21	0.38
WI1	Wood Ctr	Mixed	45	12	81	44	0.76	0.42
WI1	Post Off	100% Comm	12	0	100	54	0.90	0.19
WI1	Rustler	100% Comm	12	0	100	39	0.79	0.19
NY3	Southgate	100% Comm	179	2	21	13	0.20	0.28
WI1	State Fr	74% Comm	29	10	77	27	0.62	0.24
MI1	Indus. Dr	100% Ind	63	0	64	18	0.10	0.71
MI1	Grace S.	52% Ind	75	5	39	20	0.11	0.43

The site mean EMC values are useful but the loading values are usually what get published in the literature. Using the event mean concentrations and assuming 100 events per year, annual loads from the Great Lakes projects were calculated by the NURP team. We used 100 events per year basically because it simplified our analysis. The number of storm events that occur annually in the Great Lakes Basin, however, ranges from 90 to 120. Loadings from the NURP Great Lakes sites are summarized in Table 3.

TABLE 3. LOADINGS FROM NURP GREAT LAKES SITES

Pollutant	Mean event load (kg/ha)	Annual load (kg/ha/yr)
Total suspended solids	4.7658	477
Total phosphorus	0.007836	0.78
Soluble phosphorus	0.000979	0.10
TKN	0.037096	3.7
NO_2 + NO_3	0.019934	2.0
Total copper	0.000597	0.06
Total lead	0.004245	0.42
Total zinc	0.006399	0.64

Table 3 includes all land uses taken together. We found, at the 95% confidence level, that there is no significant difference in pollutant loading values between the various land uses.

The Nonpoint Source Control Task Force of the IJC also published loading values based on their pilot watershed studies. Table 4 shows that the NURP numbers compare well with the PLUARG data.

Annual urban runoff loads for total phosphorus from the entire Great Lakes Basin were compared between the two studies. Both showed that urban runoff accounted for approximately 7% of the nonpoint load and 3% of the total load.

ASSESSMENT OF CONTROL PRACTICES IN GREAT LAKES BASIN

In addition to assessing the concentrations of urban runoff, the Michigan, Illinois, and Wisconsin projects all evaluated the effectiveness of either street sweeping or detention basins. In fact, control practice evaluation was the focus of these projects. Milwaukee, Wisconsin, evaluated street sweeping. The primary purpose of this project was to evaluate the potential improvement in stormwater quality caused by an accelerated street sweeping program. The project selected pairs of small, homogeneous watersheds. One basin served as the test area and one as the control area. The control area was swept using the same baseline frequency at which it had customarily been

TABLE 4. SUMMARY OF RANGES OF UNIT AREA LOADS OF SELECTED MATERIAL BY LAND USE FROM PILOT WATERSHEL STUDIES DONE BY PLUARG

Land uses	Annual unit area loads (kg/ha/yr)							
	Suspended solids	Total phosphorus	Filtered reactive phosphorus	Total nitrogen	Lead	Copper	Zinc	Chlorine
I Rural								
General agriculture	3-5600	0.1-9.1	0.01-0.6	0.6-42	0.002-0.08	0.002-0.09	0.005-0.3	10-120
Cropland	20-5100	0.2-4.6	0.05-0.4	4.3-31	0.005-0.006	0.014-0.064	0.026-0.083	10-50
Improved pasture	30-80	0.0-0.5	0.02-0.2	3.2-14	0.001-0.015	0.021-0.038	0.019-0.172	-
Forest/wooded	1-820	0.02-0.67	0.01-0.10	1-6.3	0.01-0.03	0.02-0.03	0.01-0.03	2-20
Idle/perennial	7-820	0.02-0.67	0.01-0.07	0.5-6.0	0.01-0.03	0.02-0.03	0.01-0.03	20-35
Sewage sludge	-	0.02	0.01	11	0.01	0.005	0.2	10
Waste water spray Irrigation	-	0.2-1.4	0.1-1.3	2-2.37	-	-		40-160
II Urban								
General urban	210-1750	0.3-2.1	0.05-0.3	6.2-10	0.14-0.5	0.05-0.13	0.3-0.6	130-380
Residential	620-2300	0.4-1.3	0.2	5-7.3	0.06	0.03	0.02	1.050
Commercial	50-830	0.1-0.9	0.02-0.08	1.9-11	0.17-1.10	0.07-0.13	0.25-0.43	10-150
Industrial	400-1700	0.9-4.1	0.3	1.9-14	2.2-7.0	0.29-1.3	3.5-12.0	-
Developing urban	27.500	23	0.1	63.0	-	-	-	75-160

been swept. The test areas were swept at frequencies that were higher than in the control areas. The accelerated sweeping frequencies were selected to represent the possible range of sweeping frequencies that might be socially and economically acceptable.

A summary of street sweeping performance for the two Wisconsin basins is shown in Figure 2.

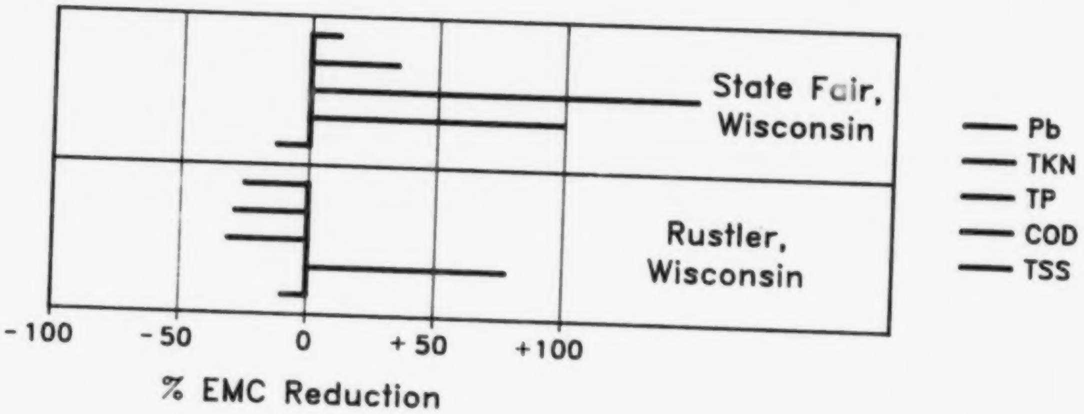


Figure 2. A summary of street sweeping performance.

As shown in the figure, the mean concentrations are as likely to be increased as decreased by street sweeping. Also, it is shown that street sweeping never produced a dramatic (i.e., >50%) reduction in concentrations.

The analysis of the NURP street sweeping data base indicated that there is generally no significant difference between median EMCs for swept and unswept conditions. This is shown in Figure 3.

Ann Arbor, Michigan, Lansing, Michigan, and Glen Ellyn, Illinois evaluated the effectiveness of detention basins. The basins monitored were "wet" basins--that is, they maintain a permanent pool of water. The ponds were designed such that runoff from an individual storm displaces all or part of the prior volume, and the residual is retained until the next storm event.

The data from the basins were analyzed and it was determined that the basins were fairly effective in removing pollutants. The performance efficiency was determined on the basis of the total pollutant mass removed over all storms. Table 5 summarizes detention basin performance in terms of reduction in pollutant mass loads over all monitored storm events. The analysis methodology used suggests that performance should be expected to improve as the overflow rate (QR/A=mean runoff rate/basin surface area) decreases and as the volume ratio (VB/VR=basin volume/mean runoff volume) increases. The basins in Table 5 are listed in increasing order of expected performance capabilities.

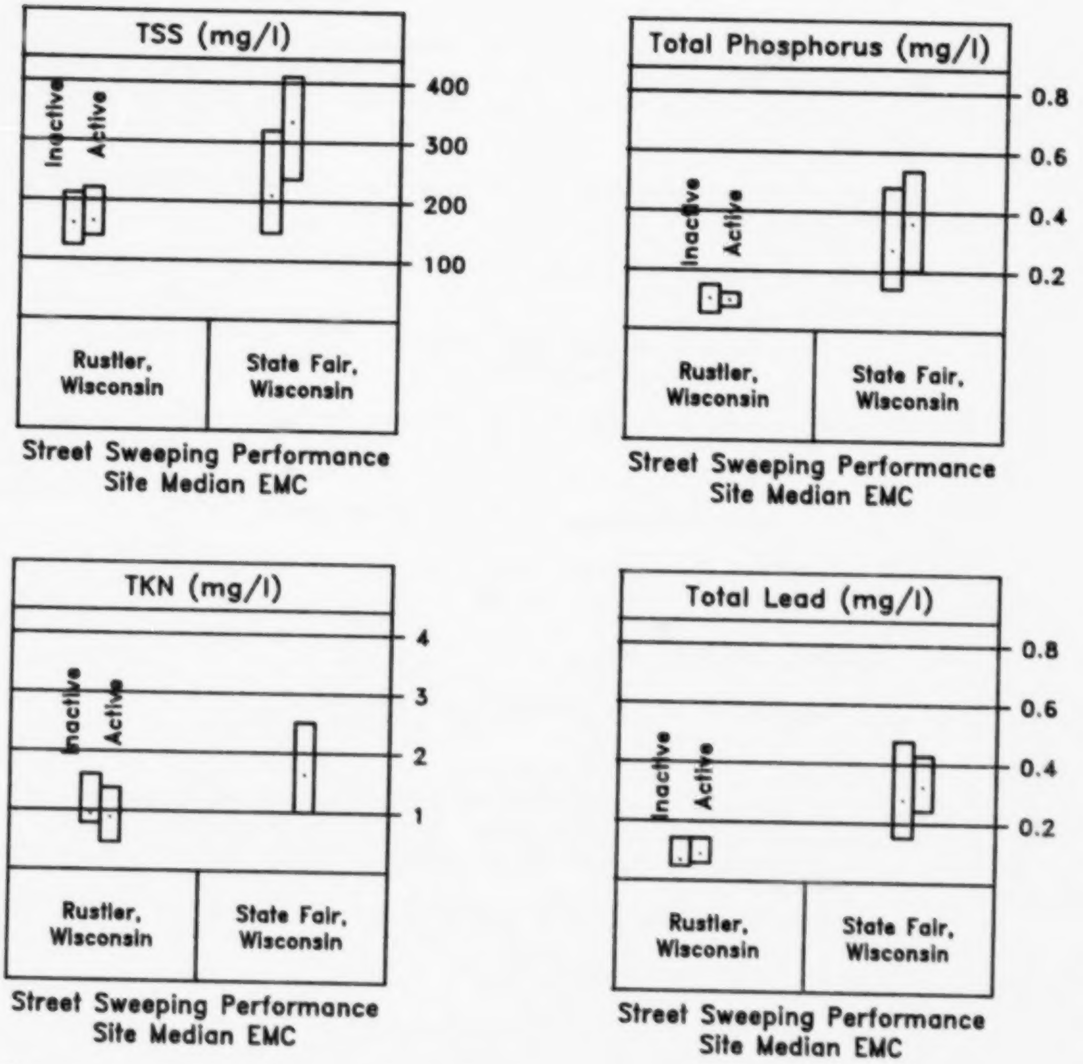


Figure 3. NURP street sweeping data.

An alternative approach for characterizing the performance of detention basins was developed by the NURP team. This approach concentrates on the variable characteristics of individual storm events and how these are modified by the detention device. A comparison of the mean and coefficient of variation of basin inflow and discharge concentrations provides a measure of the performance of the device.

Tables 6 and 7 provide a summary of detention basin performance in the Great Lakes Basin when assessed in this manner. In most cases, more inlet storm events were monitored than discharge events, and some inlet events do not

TABLE 5. DETENTION BASIN PERFORMANCE

Pollutant and site	No. of storms	Size ratios		Average mass removals-all monitored storms (percent)*									
		QR/A	VB/VR	TSS	BOD	COD	TP	Sol.P	TKN	NO 2+3	T. Cu	T.Pb	T. Zn
Lansing Grace St. N.	18	8.75	0.05	-	14	-	-	-	-	-	-	9	-
Lansing Grace St. S.	18	2.37	0.17	32	3	-	12	23	7	1	-	26	-
Ann Arbor Pitt-AA	6	1.86	0.52	32	21	23	18	-	14	7	nm	62	13
Ann Arbor Traver	5	0.30	1.16	5	-	15	34	56	20	27	nm	nm	5
Ann Arbor Swift Run	5	0.20	1.02	85	4	2	3	29	19	80	nm	82	-
Lansing Waverly Hills	29	0.04	7.57	91	69	69	79	70	60	66	57	95	71
NIPC Lake Ellyn	23	0.10	10.70	84	nm	nm	34	nm	nm	nm	71	78	71

*- indicates apparent negative removals, nm indicates pollutant was not monitored.

have a matching discharge event and vice-versa. For the larger basins where storm inflow displaces only a fraction of the basin volume, it is unlikely that influent and effluent for a specific event represent the same volume of water. The assumption used in this analysis is that the inflow events that were monitored provide a representative sample of the total population of all influent EMCs. Similarly, it is assumed that the monitored effluent events are a representative sample of all basin discharge EMCs.

TABLE 6. DETENTION BASIN PERFORMANCE*

Project and site	No. of storms	Percent reduction in mean EMC†									
		TSS	BOD	COD	Tot.P	Sol.P	TKN	NO2+3	Tot.Cu	Tot.Pb	Tot.Zn
Lansing Grace St. N.	23/20	(6)	(26)	15	(10)	(26)	11	(1)	(9)	39	(9)
Lansing Grace St. S.	18/17	22	4	(3)	6	0	(5)	(20)	25	14	7
Ann Arbor Pitt-AA	6/6	38	17	23	28	(2)	11	8	n	59	22
Ann Arbor Traver	5/5	0	(66)	12	37	63	19	28	n	n	19
Ann Arbor Swift Run	5/5	83	11	(3)	(38)	21	25	77	n	86	n
Lansing Waverly Hills	35/30	87	52	52	69	56	30	54	53	93	58
NIPC Lake Ellyn	25/20	92	n	64	61	62	n	82	88	91	87

*In/Out: numbers are approximate and vary with pollutant. Removals in parentheses indicate negative removal.

†"n" indicates pollutant either not monitored or number of observations is too small for reliable estimate of percent reduction.

For each basin influent and effluent, the arithmetic mean and variance were computed based on the relationships for lognormal distributions. The percent reduction in the mean concentration and the coefficient of variation are tabulated.

Performance characteristics are generally consistent using either approach. Performance improves with detention basin size relative to catchment size. (It is important to note that the Ann Arbor-Traver site had an unstabilized bank at the outlet of the newly constructed basin. This accounts for the poor suspended solids removal.)

TABLE 7. DETENTION BASIN PERFORMANCE*

Project and site	No. of storms	Percent reduction in coeff. of var. of EMCs									
		TSS	BOD	COD	Tot.P	Sol.P	TKN	NO2+3	Tot.Cu	Tot.Pb	Tot.Zn
Lansing Grace St. N.	23/20	14	49	35	(7)	(13)	30	0	0	45	(31)
Lansing Grace St. S.	18/17	(7)	(59)	39	13	0	20	21	17	18	15
Ann Arbor Pitt-AA	6/6	17	(6)	10	28	(84)	37	0	n	53	(5)
Ann Arbor Traver	5/5	14	(109)	58	(3)	42	(150)	(82)	n	n	0
Ann Arbor Swift Run	5/5	(5)	39	50	(150)	0	20	(150)	n	26	n
Lansing Waverly Hills	35/30	38	5	69	34	26	(8)	(198)	(22)	34	(36)
NIPC Lake Ellyn	25/20	44	n	41	71	48	n	(115)	60	19	41

*In/Out: numbers are approximate and vary with pollutant. Removals in parentheses indicate negative removal.

†"n" indicates pollutant either not monitored or number of observations is too small for reliable estimate of percent reduction.

The urban component of nonpoint sources received relatively little attention in the PLUARG studies. The PLUARG studies centered mainly on problem characterization, watershed planning, technology demonstrations and policy development. Therefore, it is not possible to compare the results of the control practice evaluation done in NURP with the IJC Nonpoint Source Task Force evaluation.

CONCLUSIONS

The NURP team, with the assistance of the technical consultants, analyzed and summarized the data from 22 of the 28 participating projects. Five of these projects were located in the Great Lakes Basin.

The data from the NURP projects in the Great Lakes Basin consisted of assessment data to help determine the existence of water quality problems caused by urban runoff and best management practice data to determine the effectiveness of control practices.

Examination of the assessment data from the Great Lakes projects shows that the loading values for the studied pollutants are relatively low. The numbers calculated for all the pollutants were lower by at least 15% than the nationwide values reported in the other NURP projects.

The NURP team focused on total phosphorus loads from the Great Lakes Basin and found that urban runoff accounted for 7% of the nonpoint load to the lakes and 3% of the total load. PLUARG reported similar loading values.

Thus, based on an analysis of the NURP data, urban nonpoint sources do not constitute a major portion of the pollutant load to the Great Lakes Basin. Pollutants in urban runoff, however, may contribute to water quality problems in the local receiving waters and near shore areas of the Great Lakes.

The PLUARG report also concluded that pollutant loadings from urban runoff generally do not constitute a significant problem on a lakewide basis.

Two control practices were evaluated in the Great Lakes Basin--street sweeping and detention basins. Analysis of the NURP street sweeping data showed that no significant reductions in event concentrations were realized by street sweeping. In no case did reductions in concentration ever exceed 50%. It can be concluded that street sweeping is generally ineffective as a technique for improving the quality of urban runoff.

Most of the detention basins monitored in the Great Lakes area were wet basins--that is, basins that maintain a permanent pool of water. The basins monitored were designed such that runoff from an individual storm displaces all or part of the prior volume, and the residual is retained until the next event. Analysis of the detention basin data in the Great Lakes area generally showed that they were effective in removing most pollutants. It was found that performance improved with detention basin size relative to catchment size and hence the magnitude of the runoff processed. Reduction in overall mass load ranged from negative to greater than 90% removal.

TRENDS IN THE DEVELOPMENT OF WATER TOXICOLOGY IN THE USSR AND THE US

by

L. A. Lesnikov[1]

ABSTRACT

Studies of the chronic effects of toxicants on fish in the Union of Soviet Socialist Republics and the United States are reviewed and maximum permissible concentrations and water quality criteria of the respective countries are compared.

INTRODUCTION AND DISCUSSION

The problem of contamination has acquired urgency in connection with the swift growth of industry and urbanization of the population (Klein 1957). Water toxicology--the science of the influence of contaminants on water organisms--began to develop. In the USSR, the first toxicological works concerned estimates of the effect of petroleum contamination on fish (Arnold 1897, Nikolskiy 1983, Khlopin 1902, Chermak 1986), and in Great Britain--of the salts of heavy metals (Carpenter 1927). According to "Quality Criteria for Water" (Anon. 1972), the study of the effect of contaminants on fish was begun from the beginning of the century (Marsh 1907, Shelford 1917).

In our country, the dependence of the survival rate of test organisms on the concentration of the substance being studied was analyzed on the basis of brief experiments (Beklemishev and Lyubishchev 1924). In our opinion (Lesnikov 1976), this direction is most promising. In the United States, the basic indicator in estimating the effect is the establishment of median lethal concentrations--24, 48, and 96-hour LC_{50} (Anon. 1979, Anon. 1976). Along with this, proposals to establish LC_{50} for a number of periods and only then analyze the shape of the curve are encountered (Alabaster and Lloyd 1980, Anderson 1948). Probably, both curves should be identical within the limits of statistical accuracy (Figure 1). Anderson (1948) proposes considering the value of LC_{50}. Alabaster and Lloyd (1980) consider the asymptote toward which this segment of the curve strives as the threshold of the median concentration.

---

[1] USSR State Scientific Research Institute of Lake, River and Fishing Management, Leningrad, USSR.

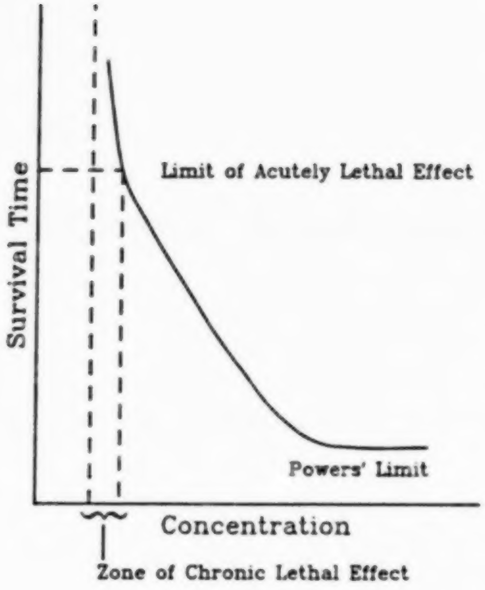


Figure 1. Dependence of survival time on toxicant concentration.

The bend of the curve is considered as the limit of acute toxicity on the curve of median lethal time (Lesnikov 1976), and it has been proposed (Stroganov 1971) that the asymptote be taken as the limit of lethal effect, obviously chronic, if the duration of the experiment is sufficient.

In our country, the chronic effect of toxicants on fish was studied at the end of the century (1893); the works of N. B. Yel'tsina (1939) and N. S. Stroganov (1941) are widely known. A schematic diagram to determine the maximum permissible concentrations (MPC) of contaminants for fishing industry basins has been created (Lesnikov 1973). This diagram includes the conduct of studies on the effects of substances on the hydrochemical condition of the basins, algae, zooplankton, zoobenthos, and fish (roe, larvae, mature individuals). Periods for the duration of the experiments have been adopted: hydrochemical and microbiological--1 month; on algae and water plants--1 month; on invertebrates--1 month (subacute), up to 3 months (chronic); on fish (with roe and larvae) from fertilization to the start of active feeding of the larvae (or resorption of the yolk sac); on adult fish--up to 3 months (subacute), up to 6 months (chronic).

In the United States, they also changed over to the conduct of chronic experiments from the beginning of the 1970's (Brungs 1972). Up to now there are data on the long-term effect of cadmium (Benoit et al. 1976); carbaril (sevin) (Carlson 1972); chlordane (Carwell et al. 1972); copper (Pickering et al. 1977); methylmercuric chloride and lead (Christensen et al. 1977, McKim et al. 1976); toxaphene (Mayer et al. 1975); phthalic esters (Sanders et al. 19--); Bayer-73 (Sanders 1977); acrolein, heptachlor, endosulfan, and trifluralin (Macek et al. 1976); chromium (Sauter et al. 1976); arochlor-1254 (Snarski and Puglisi 1976); diazinon (Allison and Hermanutz 1977); and hydrogen sulfide (Lloyd 1976). Obviously, the list of substances with which chronic experiments were conducted in the United States is far from complete.

In the USSR, up to now about 500 fishing industry MPC's have been developed that are based on the results of chronic experiments.

We examine fishing industry MPC's in the USSR and "Quality Criteria for Water" (Anon. 1976) which protect the hydrobionts in the United States since these are the standards. According to our data, they are close to those of public health and, in 60 percent of the cases, below them. Therefore, in protecting the fish-producing properties of basins, we also protect other types of water consumption. Protection of fishing industry interests is not a special point of little significance in the overall strategy of protecting basins from contamination.

To what degree are the procedures for working out the MPC's in the USSR and the water quality criteria in the United States comparable? Some of the test organisms used in both countries are identical. They are the fish--Salmo irrideus, Cyprinus carpio, Esox lucius, and Perca fluviatilis; the invertebrates-- Daphnia magna, Chironomus plumosus, and Asellus aquaricus as well as various species of Gammaridae; and algae--Scenedesmus quadricanda.

A number of species are similar in toxicological resistance (Lesnikov 1980). Therefore, the data obtained in both countries are completely comparable. A comparison of the MPC values and the water quality criteria shows that, as a rule, the figures are either identical or close to one another (Table 1).

A comparison of the values obtained shows that in the United States greater significance is devoted to the consideration of local conditions while in the USSR values common for the country are established. The latter may be excessively rigid for some cases but more convenient for monitoring purposes and more economical as regards calculations of water protection systems. A zero MPC is adopted in the USSR if the MPC of the substance is below 0.0001 mg/l.

In the USSR one of the mandatory indices is the estimate of the effect of contaminants on the hydrochemical condition of basins (the pH dynamics, dissolved oxygen, biological oxygen requirement, permanganate oxidizability, forms of nitrogen). We did not find similar studies in accessible literature from the United States.

In turn, a procedure has been developed in the United States to estimate the effect of substances on the spawning of fish, which permits considering the effect of toxicants on the maternal species, on their progeny from roe to spawning, and on the roe and larvae from them. Unquestionably,

TABLE 1. WATER QUALITY CRITERIA (WQC) OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY (Anon. 1976) IN COMPARISON WITH CORRESPONDING FISH INDUSTRY MPC's OF THE USSR (mg/l)

Contaminants	U.S.		USSR	
	WQC	Notes	MPC	Notes
Ammonia	0.02	nonionized, e.g., ammonia gas	0.05	ammonia gas
			0.5	ammonia salt
Arsenic	0.05		0.005	
Cadmium	0.0004	for salmon; in soft water,	0.005	in fresh water
		for other species		
	0.004	in soft water		
	0.012	in hard water		
	0.005	in sea water	0.01	in sea water
Chromium	0.1		0.001	
Copper	0.1 96 h LC_{50}	for most sensitive species	0.001 0.005	in fresh water in sea water
Cyanides	0.005		0.05	
Mercury	0.00005	in fresh water	0.0001	mercuric chloride in fresh water
			0.00001	granosan in fresh water
	0.0001	in sea water	0.001	in sea water
Nickel	0.01 96 h LC_{50}	for most sensitive species	0.01	

(continued on next page)

Table 1. (Continued)

Contaminants	U.S.		USSR	
	WQC	Notes	MPC	Notes
Petroleum products and lubricating oils	0.01 96 h LC_{50}	for several most sensitive species: film on water surface and in soils prohibited; commercial qualities of hydrobionts should not spoil (no exact figures)	0.05 0.01	for fresh water film on water surface prohibited for sea water
Dissolved oxygen	5.0 and more	in water and soils of salmon spawning grounds	6.0 and more	in summer in water
			6.0	in winter in 1st category basins
			4.0	in winter in 2d category basins
DDT	0.000001			absence of any preparative forms
Lindane	0.00001	for fresh water		absence of all forms
-isomer of hexachloran	0.000004	for sea water		and isomers of hexachloran
Malathion (karbofos)	0.0001			absent
Toxaphene (Polychlorocamphene)	0.000005			absent

(continued on next page)

Table 1. (Continued)

Contaminants	U.S.		USSR	
	WQC	Notes	MPC	Notes
pH	6.5-9.0 6.5-8.5	for fresh water for sea water	6.5-8.5	
Phenol	0.001	to protect commercial quality of fish	0.001	
Phosphate Phosphorous	0.1	for river sections far from lakes	0.04	for K_2HPO_3 (0.31 for salt)
	0.05	for rivers near lakes		
	0.025	for lakes and reservoirs; in addition, the charge for basins is calculated		
Phthalic esters	0.003		0.05	phthalic anhydride
Zinc	0.01 96 h LC_{50}	according to sensitivity of local species	0.01 0.05	for fresh water for sea water

these are important and needed studies. In the USSR, the introduction of such procedures is hindered in that our country is located further north. The fish have relatively long life cycles (4 to 7 years or more); therefore, such experiments are unrealistic. However, we are now selecting species with a relatively short life cycle and sufficiently sensitive to contaminants. Such experiments on invertebrates and Daphnias have long been conducted in the USSR. It is shown on fish that if the producers (male or female or one of the parents) are in a toxic solution during the last month of ovogenesis the viability of the progeny is reduced noticeably (Bykova 1978). The experiments were performed on lamprey (Misgurnus fossilis).

A common scheme for the conduct of experiments to estimate the danger of contaminants in the U.S. is close. Therefore, cooperation of efforts and the exchange of data are fully realistic. At present there are about 650 substances on which various toxicological data are available in the

USSR and the US or other countries. Now tens of thousands of contaminants are entering the basins. The conduct of toxicological studies according to the full program requires from one to three and a half years. Therefore, cooperative efforts of Soviet and American water toxicologists is not only desirable, but also necessary to accelerate the process of compiling a "toxicological catalog" of at least the basic contaminants.

REFERENCES

Alabaster J. S. and R. Lloyd. 1980. Water Quality Criteria for Freshwater Fish. London; Boston. 297 p.

Allison D. T. and O. Hermanutz. 1977. Toxicity of Diazinon to brook trout and fathead minnows. U.S. Environmental Protection Agency, Washington D.C. EPA-600/3-77-060.

Anderson B. G. 1948. The apparent threshold of toxicity to Daphia magna for chlorides of different metals when added to Lake Erie water. Trans. Amer. Fish. Soc. 78:96-113.

Anon. 1976. Quality Criteria for Water. U.S. Environmental Protection Agency, Washington D.C. 256 p.

Anon. 1979. A review of the EPA Red Book: Quality Criteria for Water. Bethesda, MD. 313 p.

Arnold, I. N. 1897. On the effect of petroleum on fish. Vestnik Rybopromyshlennosti. 12(4):167-196.

Beklemishev, V. N. 1923. On the course of the extinction of the entomostraca population in a salt solution. Trudy pervogo Vserossiyskogo syezda zoologov, anatomov, i gistologov [Proceedings of the First All-Russian Congress of Zoologists, Anatomists, and Histologists]. pp. 142-143.

Beklemishev, V. N. and A. A. Lyubishchev. 1924. Formulas to find constants in toxicity equations. Izvestiya Biologicheskikh Nii I Biologicheskikh Statyey Permskogo Universiteta. 3(4):41-52.

Benoit D.A., E.N. Leonard, G.M. Christensen and J.T. Fiandt. 1976. Effects of cadmium on three generations of brook trout (Salvelinus fontinalis). Trans. Amer. Fish. Soc. 105(4):550-556.

Brungs, W.A. 1972. Effects of pesticides and industrial wastes on surface water use. In: River Ecology and Man. N.Y. p. 353-365.

Bykova, A.V. 1972. Toxic effect of mercuric chloride on embryogenesis of fish using the example of lamprey. Express information service of Central Scientific-Research Institute of Information and Technical-Economic Studies of the Fishing Industry. Issue 9. pp 11-13.

Carlson, A.R. 1972. Effects of long term exposure to carbaril (sevin) on survival, growth and reproduction of the fathead minnow (Pimephales promelas). J. Fish. Res. Board Canada. 28(5):583-587.

Carpenter, K.E. 1927. The lethal action of soluble metallic salts on fishes. Brit. J. Exper. Biol. (4):278-390.

Carwell, R.D., D.G. Foreman, T.R. Payne and D.J. Wilbar. 1977. Acute and chronic toxicity of chlordane to fish and invertebrates. U.S. Environmental Protection Agency, Washington DC. EPA-600/3-77-019.

Chermak, N. 1986. On the effect of petroleum on fish. Vestnik Rybopromyshlennosti. 1:1-30.

Christensen G., E. Hunt and J. Fiandt. 1977. The effect of methylmercuric chloride, cadmium chloride and lead nitrate on six biochemical factors of brook trout (*Salvelinus fontinalis*). Toxicology and Applied Pharmacology. 42:523-530.

Khlopin, G.V. 1902. Contamination of running water by farm and factory waste products and measures for its elimination. Ucheniye zapisky Yuryevetskogo Universiteta (Scientific Notes of Yurevets University). No 2. p 115.

Klein, L. 1957. Aspect of River Pollution. 621 p.

Lesnikov, L.A. 1973. Methodological Instructions on Establishing Permissible Concentrations of Harmful Substances in the Water of Fishing Industry Basins. Leningrad, USSR. 22 pages.

Lesnikov, L.A. 1976. A comparison of various procedures for the conduct of water toxicological experiments. Izvestiya Gosniorkh. 109:3-7.

Lesnikov, L.A. 1980. Comparison of principles of development and use of water quality standards in the USSR and the USA. U.S. Environmental Protection Agency, Washington DC. EPA-600/9-80-034.

Lloyd, L.S. 1976. Chronic effects of low levels of hydrogen sulfide on freshwater fish. U.S. Environmental Protection Agency, Washington DC. EPA-600/3-78-076.

Lloyd, L.S., D.M. Oseid D.M., I.R. Adelman and Broderius. 1976. Effect of hydrogen sulfide and invertebrates. Part 1. Acute and chronic Toxicity Studies. U.S. Environmental Protection Agency, Washington DC. EPA-600/3-76-062.

Macek, K.J., M.A. Lindberg, S. Sauter, K.S. Buxton and P.H. Costa. 1976. Toxicity of four pesticides to water fleas and fathered minnows. Acute and chronic toxicity of acrolein, heptachlor, endosulfan, and trifluralin to the water flea (*Daphnia magna*) and and the fathead minnow (*Pimephales promelas*). U.S. Environmental Protection Agency, Washington DC. EPA-600/3-76-899.

Marsh, M.C. 1907. The effect of some industrial wastes on fishes. Water supply and irrigation paper. N 192, p. 337-348.

Mayer, F.L., P.M. Mehrle and W.P. Dwyer. 1975. Toxaphene effects on repro-reproduction, growth, and mortality of brook trout. U.S. Environmental Protection Agency, Washington DC. EPA-600/3-75-013.

McKim, J.M., G.F. Olson and G.W. Holcombe. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (Salvelinus fontinalis). Toxicity, accumulation, distribution and elimination. J. Fish. Res. Board Canada. 33(12):2726-2734.

Nikolskiy, A.M. 1893. On the significance of petroleum in the life of fish of the Volga River. Rybnoye Delo. 15:260-288.

Pickering G., W. Brungs and M. Gast. 1977. Effect of exposure time and copper concentration on reproduction of the fathead minnow (Pimephales promelas). Water Research. 11:1079-1083.

Sanders, H.O. 1977. Toxicity of the molluscocide and residue dynamics of Bayer 2352 in aquatic invertebrates. Investigation in Fish control. 78:1-7.

Sanders, H.O., F.L. Mayer and D.F. Walsh. 19--. Toxicity, residue dynamics and reproductive effects of phthalate esters on aquatic invertebrates. Environmental Res.. 6(1):84-90.

Sauter S., K.S. Buxton, R.J. Macek and S.R. Petrocelli. 1976. Effects of exposure to heavy metals on selected freshwater fish. Toxicity of copper, cadmium, chromium and lead to eggs and fry of seven fish species. U.S. Environmental Protection Agency, Washington DC. (EPA-600/3-76-105).

Shelford, V.E. 1917. An experimental study of the effects of gas wastes upon fishes with especial reference to stream pollution. Bull. Illinois State Lab. for Nat. History. 11:381-412.

Snarski, V.M. and F.A. Puglisi. 1976. Effects of arochlor or 1284 on brook trout (Salvelinus fontinalis). U.S. Environmental Protection Agency, Washington DC. EPA-600/3-76-112.

Stroganov, N.S. 1941. New ways to solve the problem of the effect of industrial runoff water on water organisms. Scientific notes of Moscow State University. Biologiya. Issue 60. pp 5-24.

Stroganov, N.S. 1971. A procedure for determining toxicity of the water Medium. In: Metodiki issledovaniy po vodnoy toksikologii (Procedures for Studies on Water Toxicology). Moscow, USSR. pp 14-60.

Yeltsina, N.V. 1939. The effect of sea salt on the development of daphnia and adaptation to conditions of increased salinity. Problems in Ecology and Biocenology. Issue 4. pp 128-142.

U.S. GOVERNMENT PRINTING OFFICE 19860646-116-40649